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ANNALS OF BOTANY

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Cytology of *Osmunda* and *Doodia*.

II. On the Gametophyte and Post-meiotic Mitoses in the Gametophytic Tissue of *Doodia*.¹

BY

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With Plates I-IV.

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INTRODUCTION.

THE present study of the post-meiotic chromosomes of *Doodia* was undertaken not only to test the author's view with respect to the period of splitting of chromosomes in post-meiotic mitoses, but also, by determining more accurately the exact nature of the transformation of the chromosomes in the post-meiotic telophase of the gametophytic tissue, to

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ascertain to what extent, if at all, this transformation will serve to throw any light on the interpretation of the heterotype prophase. The present paper is thus not only intended to supplement the earlier one of this series (75), but serves to illustrate the constancy of certain phenomena described in the former paper. It should, however, be borne in mind that our present knowledge of the prothalli from the cytological point of view is far less complete than that of the sporophyte plant, and the cogency of some of the important conclusions of the chromosome cycle lies in the careful observation and critical study of the phenomenon which takes place in the gametophytic tissue. To do so adequately would require a very careful study of the post-meiotic mitoses which will complete the evolutionary story of the sexuality of such higher types of vegetation as Ferns. No doubt, sexuality is the common property of the animals and plants alike, but so far as our present knowledge of ferns is concerned, sexuality is a condition of meiosis, and meiosis in its turn makes possible the continuance of sexual act. It is proposed in the present paper to attempt a detailed study of the post-meiotic (gametophyte) stage, because it intervenes between meiosis and sexual fusion. In order to give an uninterrupted account of the cytological phenomena and history of the chromatin through the critical stages, attention has been particularly focused on the following points:

1. The nature of the double spireme in the post-meiotic mitoses of *Doodia aspera*, and
2. The significance and development of the longitudinal fission with special reference to the bearing of the observed facts on the problem of chromosome reduction.

As *Doodia* is easy to manipulate, not only because the tissues are easily 'fixed' and the nuclei stain readily, but also because it lends itself admirably to cytological study, it was suggested by Professor Farmer that it would repay further research. In order to get a critical and detailed knowledge of the cytology of *Doodia*, it has been found expedient to study the post-meiotic division, so that the visible mode of transition from telophase to early prophase could be followed. The significance of this phenomena, namely, the disorganization of chromosomes and the gradual distribution of their substance in the nucleus and the ensuing reconcentration of their elements, is one of fundamental importance, and will be taken up in some detail in the discussion.

STATEMENT OF THE PROBLEM.

It is useless to give again an extensive review of all the literature concerning this problem. Such a review has been published many times, and a fairly extensive *résumé* of researches in this particular branch of study was presented in Part I of this paper (75). It is most important to know

definitely at *what stage* and in *what manner* the splitting of chromosomes—around which centre all the events of mitoses—takes place. A very accurate knowledge of the events in the relatively simple mitoses of the gametophytic tissue is necessary, as without it a full understanding of the complex and significant phenomena of maturation is rather difficult to follow. I shall be content to give a very general idea, briefly, in so far as plant chromosomes are concerned, of the different points principally in dispute, and shall critically examine the ideas prevalent on chromosomic evolution with special reference to the time of division and the mechanism of this division.

I. The evolution of the chromosome with reference to the time of division.

(a) According to the conception of a first group of authors, such as Grégoire and Wygaerts (38), Grégoire (39), Martins Mano (52), Sharp (79, 80), R. de Litardière (46), &c., the daughter chromosome becomes hollow at the telophase or at the anaphase, and a system of vacuoles develops which subsequently leads to a spongy alveolar or reticular structure. The juxtaposition of the different chromosomes thus transformed is said to result in the making of a resting nucleus of a 'réseau de réseaux' (lit. network of networks) type. At the beginning of the prophase, a concentration transforms each alveolar chromosome into a fine and undivided chromatic filament. It is this fine filament which undergoes very rapid longitudinal division, this division therefore appearing at the beginning of telophase. Němec (62), Müller (59), &c., also report that the vacuolation of the chromosomes does not begin until telophase, the initiation of which may begin during anaphase.

(b) The transformations which one observes at the telophase have for a second group of authors a very different significance.

To Bonnevie (9, 10, 11, 12) the condensation in the telophasic chromosome of the peripheral bed of chromatin brings about the differentiation in each of them of one or two long and fine endogenous spiral filaments, the whole of the rest of the chromosome is abandoned to the nuclear sap, and at the same time all the spirals anastomose among themselves to form the network of the resting nucleus. At the prophase, all the spiral filaments individualize afresh, and undergo a process of contraction and condensation which brings them, undivided, up to the end of the prophase. Bonnevie notes, however, in certain cases, a duality, a little more precocious, before this period of condensation, but does not observe it later, and doubts if the halves which seem to result from this first cleavage correspond to the two future daughter chromosomes. The essential stage of the division, according to her, only occurs at the equatorial plate following the preliminary division in the longitudinal axis of the chromosome. So, according to Bonnevie the chromatic material forms a spiral thread within the chromosome

during the telophase which uncoils and emerges from the chromosome in the following prophase.

Vejdowsky (87), who has, however, only studied animal cells, arrives at conclusions which agree in part with the preceding ones, and in part differ from them. According to this author, a spiral chromatic fibre appears at the periphery of the anaphasic chromosome. Subsequently, this spiral fibre or 'chromonema' becomes achromatic, supporting however a certain number of chromatic granules. Anastomoses are formed between the fibres thus transformed, but disappear at the prophase. It is then that the chromatic granules unite in such a manner, end to end, as to constitute a new 'chromonema', which afterwards undergoes division at the end of the prophase.

It is interesting to note that Vejdowsky holds that the small 'droplets' which are found in the chromosome complex are really vacuoles within the chromosome. According to his view the nuclear membrane represents the peripheral portions of certain chromosomes.

Frisendahl (34) observes in *Myricaria* that each of the chromosomes is represented throughout the rest by four (rarely more) parts arising by a true split and further subdivision during telophase.

Recently, however, a new view of the transformation which one observes at the telophase is presented by Martens (50) for *Paris quadrifolia*. He observes two constituents, one 'chromonematique' which may be translated as 'chromonema', a zigzag thread which is easily distinguished at all times, and another achromatic ground substance surrounding the chromonema and impregnated with nucleolar matter. The appearance of vacuolation is really due, according to Martens (50), to this zigzag thread, which elongates and becomes thin during prophase; it does not split at this stage. By a series of convolutions a flat ribbon is formed, and, owing to the thinning out of the middle portions lying between the edges of the ribbon-like convoluted band of chromonema substance, a separation into two longitudinal halves occurs in the chromosome *anlage*. This account, in effect, would seem to imply the disposition, on the two sides of the ribbon, of chromatic particles that originally were arranged lengthwise in the chromonema filament. Martens believes his observations dispose of both the *alveolization* and the *longitudinal fission* theories of chromosome division, in so far as telophase and the following prophase are concerned.

Still more recently Bolles-Lee (15) contributes a paper on the structure and division of chromosomes. In a critical study of *Paris quadrifolia* he finds that the chromosomes contain a spiral periaxial filament and are surrounded by a sheath. Numerous observers have described such a spiral, while others have interpreted it as a row of alveoli. But the most surprising conclusion drawn by this observer is that the chromosomes always divide *transversely* in the telophase of the mitoses, and never longitudinally at all.

We can only say that much more convincing evidence is required before such a view would be regarded as at all probable.

(c) According to a third set of authors, the anaphasic or telophasic transformations represent neither a single alveolization nor the endogenesis of a fresh filament destined to become the chromosome of the succeeding division. They represent, on the contrary, the beginning of longitudinal division.

Strasburger (82) and Miyake (56) have shown in *Tradescantia* that a distinct process of alveolization occurs in the chromosomes during the heterotypic telophase. Farmer and Shove (31), in their critical study of *Tradescantia*, give an account of both the somatic and meiotic mitoses, and they tell us of a 'vesiculation' of the chromosomes in the somatic telophase, the chromatin becoming a 'cloud of fine granules through the linin band'; and thus describe 'broad band-like areas' with the chromatin in a dense 'granular aggregation' during the early telophase.

The telophasic transformations have been critically studied by Lundegårdh (47, 48, 49) and also by Miss Digby (25), whose admirable treatment of the subject is a very important contribution to this particular question and to the subject of meiosis generally. The idea that telophasic transformations produce in the chromosome a real chromatic duality has been defended, so far as plants are concerned, by Déhorne (22), Fraser and Snell (33), Schustow (78), Fraser (32), and Nothnagel (64).

According to the majority of these authors, the telophasic transformations tend very quickly to form two chromatic filaments which will become the two daughter chromosomes of the succeeding division. During the whole duration of rest the chromosome is therefore double, and it is found so at the succeeding prophase. In this manner the anaphase separates two halves of a chromosome whose duality dates from the telophase or from the preceding anaphase.

MECHANISM OF DIVISION.

1. Several authors (e. g. Grégoire, 38, 39) admit that the formation of two chromosomes at the expense of one is the result of a process of alveolization. According to Sharp (79, 80), the chromosomes gradually separate from each other at telophase, though they remain connected by anastomoses. The chromosomes are therefore not double during the resting stages. In the prophase the constituent reticulate chromosomes separate from one another through the breaking down of their connecting anastomoses; each gives rise to a slender chromatic thread in which later on the split develops. This split results from axial vacuolization of the simple slender thread and remains until the metaphase.

2. Bonnevie (1911) thinks division results from the preliminary bipartition of a longitudinal axis of the parent chromosome.

3. A number of other authors admit again that the division of the chromosome results from the individual bipartition of a series of the chromatic granules called chromomeres. Balbiani (3) and Pfitzner's (68) theory is that the chromatic granules visible in the nuclear reticulum arrange themselves in a series in the chromosome, and by their division cause its splitting. The chromosome which detaches itself from the resting nucleus is, according to this interpretation, an achromatic filament, the carrier of a row of chromatic granules or chromomeres. The latter, representing the elementary units of the chromosome, will each divide itself into two: according to some, before the achromatic filament is itself divided longitudinally; according to others, after this stage. These tend to join afterwards, little by little, in such a way as to give, at the end of prophase, two similar thick and uniformly chromatic chromosomes. These ideas, strongly upheld by those who explain the mechanism of heredity in terms of chromosomes, have been defended principally by Strasburger (81) and Müller (59). It is interesting to note also that Strasburger (83), Allen (1), and Mottier (57) found even the chromomere to be composed of smaller chromatic granules. More recently Wenrich (88) has established the presence, in the chromosomes of *Phryonotettix*, of chromatic granules which appear constant in number and situation.

MATERIAL AND METHODS OF INVESTIGATION.

The material on which the study of these prothalli has been based consists of three species of *Doodia*. (i) *Doodia aspera*, R. Br.; (ii) *Doodia aspera multifidia*, Hort.; (iii) *Doodia media*, R. Br., found in Australia and New Zealand. These have been grown at the Chelsea Physic Garden, under the supervision of the curator, Mr. Hales, to whom I wish to express my thanks for placing this large stock of suitable material at my disposal; without it certain facts would have been missed and others could not have been investigated so exhaustively. The large number of prothalli under observation has also cleared up certain points concerning the behaviour of chromosomes which had been met with in earlier investigations on this fern.

The spores from which the prothallia were derived were sown upon soil consisting of a mixture of vegetable mould and sand, which was thoroughly sterilized by first heating to 125° C. in a hot-air sterilizer, then to 100° C. in the steam sterilizer after it had been placed in the flower-pot, and lastly by moistening it with boiling water. The pot was covered with a glass plate, and, after it had cooled, the spores were scattered over the surface of the soil. Particular care was taken to keep the air as free as possible from floating spores; no other ferns bearing sporangia were grown in this house and no sowings were made in it. Further to guard against any other infection, the glass plates were kept constantly on the

pots. This danger was much lessened when, after a few months, the surface of the soil became closely covered with prothalli.

As a result of previous experience with similar materials, special attention was paid to fixation. Almost all the materials were fixed in the greenhouse, being at once placed in the fixing fluid, but some were brought into the laboratory as large sods and placed under a bell-jar until time of fixation. The apex of the young prothalli was cut out with scissors and the material was then put direct into the fixative. Penetration of the fixing fluid was facilitated by the use of a small air-pump, so that the prothallia sank in a few moments. Well-fixed material was also obtained by momentarily immersing the material in 35 per cent. alcohol before placing in the fixing fluid; this ensured the quick penetration of the fluid without the use of an air-pump. Owing to the delicacy of the prothalli, thorough penetration of the fixing solution was obtained without difficulty. Ordinarily the material was left in the fixing fluid from 12 to 20 hours, washed in running water from 5 to 9 hours, and then slowly dehydrated by a series of graded alcohols. It was found necessary to change the absolute alcohol at least twice.

The fixatives used mostly were strong chromic acid, acetic alcohol, corrosive sublimate, Flemming's strong and Flemming's weak solutions, and Hermann's solution. The latter gave excellent results in the present investigation. Flemming's weak solution also gave most satisfactory results, though chrom-acetic acid and 1 per cent. chromic gave results quite as satisfactory in certain cases. Another solution which was used was made up of chromic acid, 0.25 per cent., glacial acetic acid, 0.25 per cent., and water, but this was not quite as good as the former. Some remarkably good results were also obtained with acetic alcohol. Many other concentrations and solutions were also experimented with, including Schaffner's chrom-acetic acid, Juel's reagent (acetic acid, 2 per cent.; zinc chloride, 2 per cent.; 70 per cent. alcohol, 96 per cent.), and others also, but without much success.

Fixing was done as a rule about midday (chiefly between 11.30 a.m. and 1 p.m.) on bright warm days. But great difficulty was at first experienced in fixing the sexual organs, especially the archegonium and the stages of fertilization. It was surmised that penetration of the spermatozoid possibly occurred at night. Pethybridge (67) had watched penetration taking place at night. It was with this in view that the fixation of the sexual organs was done at about midnight, and this yielded better results.

From the absolute alcohol the material was placed in a solution consisting of equal parts of absolute alcohol and chloroform. Xylol was avoided, as it seemed to render the material too brittle to section. It was found best to add the chloroform gradually to the alcohol and to transfer to pure chloroform after the lapse of about three hours. Material left in pure

chloroform longer than 3 or 4 hours was in many cases rendered too brittle. The material in pure chloroform was then placed in the paraffin bath and heated to about 45° C. To this small fragments of paraffin were gradually added until a mixture of about equal parts resulted, and then the material was transferred to pure paraffin at a temperature of about 55° C., after having been in the paraffin-chloroform solution from 7 to 16 hours. It was usually allowed to remain in the pure paraffin about 24 hours, and then poured into watch-glasses and cooled in 95 per cent. alcohol, which improves the grain of the paraffin for cutting. Albumin was employed throughout for fixation of the sections to the slide.

For staining, Heidenhain's iron-alum-haematoxylin, followed by a ground stain of orange G and without a counter-stain, gave the best results. Gram's stain was also used with success. Flemming's triple stain with the following formula gave good results: Safranin, 1 per cent. solution in 50 per cent. alcohol (made up of equal parts of a 1 per cent. watery and 1 per cent. alcoholic solution), 1 minute; gentian violet, 1 per cent. solution in 1 per cent. alcohol, 5 minutes; orange G, 1 per cent. watery solution, about 22 seconds. The excess stain was wiped off and the slide was dehydrated rapidly in absolute alcohol, followed by clove oil. The clove oil was followed by cedar-wood oil, all the former being carefully removed. Breinl (safranin, methylene blue, and orange G) was also used, giving a clearer and more pleasing picture. In this way it was possible to trace the developmental history of the chromosomes, though the results obtained were checked by the use of many other dyes, used both singly and in combination. These included eosin, erythrosin, cyanin, acid-fuchsin, and methyl green.

In addition to microtome sections, observations were also made on prothalli mounted as a whole after staining with acetic carmine or borax carmine.

All the materials were embedded in paraffin melting at 54° C. Sections were cut 4–15 μ in thickness, varying with the different stages sought. Most of the sections cut at 4–7 μ thick were quite useful for critical phases. In sectioning, great care was taken in orienting the material so as to get a medium longitudinal section of the prothallus which should pass as nearly as possible through the median plane of the sexual organs.

GAMETANGIA.

The more extended and detailed our knowledge of all the stages of division, including the sexual cells of the prothallus, the better will be the chance of finding out the exact relationship of the diverse phenomena of the chromosome cycle. In the present paper only the evidence afforded by the gametophyte is considered. I reserve a critical study of the sexual

organs and embryo with special reference to their development for a later communication of this series. It may be interesting to note here that the male and female gametangia extraordinarily resemble sporangia in the fact that the gametes, like the spores, are borne internally and surrounded by a wall composed of one or more layers of cells until maturity. Like the sporangia, of which I had the opportunity of examining a large number of specimens in the previous investigation, the gametangia project more or less in a marked degree. Some, however, are deeply sunk in the parent tissue. The analogies of gametangia and sporangia will be dealt with in detail in a further study on this subject, as the species under consideration show numerous peculiarities. For the present, a few of the preliminary outlines of the male and female gametangia of this particular fern prothallus may be briefly mentioned.

THE PROTHALLUS.

The prothallus of *Doodia*, as represented in Fig. 1, is characterized by the presence of a single growing-point which is deeply seated between two more strongly growing lateral lobes; each of these consists of a single layer of cells, and the central region is more massive and is attached to the soil by numerous rhizoids (Fig. 2). Its vegetative region is larger and more strongly lobed, while the sexual region, though shorter and thicker than in the latter, is of considerable length.

In young prothalli an acropetal succession of archegonia, situated on the dorsal surface of the cushion, leads to the apex, while the antheridia, which are generally borne on the same prothallus, are scattered irregularly over the basal region of the cushion. Figs. 3 and 4 represent a median longitudinal section through the apical region of the vegetative tissues of a rapidly growing young prothallia where the nuclei are rather large and distinct and often met with in the dividing stages.

ANTHERIDIA.

The position of the antheridia on the prothallus of *Doodia aspera* is mostly superficial. The mature antheridium projects slightly from the general surface and is directed downwards. They appear generally before the archegonia and are most numerous in the basal region of the prothallus (Fig. 2). The antheridium originates as a hemispherical protrusion from a single cell of prothallus, which is then partitioned off by a transverse oblique wall. It contains cytoplasm with chlorophyll and a large central nucleus. The young antheridium consists of four cells, namely, the central mother-cell, two ring-shaped cells, and a terminal cell. The cells of the wall are provided with chloroplasts. The ripe antheridia project from the surface of the prothallus and are conspicuous by reason of the formation of

a mass of polyhedral sperm-cells or spermatocytes with dense granular cytoplasm and large nuclei (Fig. 5). In vertical section the large oval mass of spermatocytes is seen to be surrounded by a limiting layer of flattened cells (Fig. 5). If the spermatocytes are examined before the final division takes place, the nucleus is found to be large and to consist of a nucleolus. It stains deeply, while the cytoplasm remains colourless. Each nucleus is then transformed into the body of a spermatozoid. The fully matured spermatozoid shows distinctly about three complete coils in a tapering spiral (Figs. 7 and 8). Numerous cilia can be seen attached to them (Fig. 6). The wall of the antheridium consists of a single layer of cells, and shows a triangular opercular cell (Fig. 6) which breaks down to give exit to the spermatozooids. Campbell (17) also mentions this triangular operculum in *Ophioglossum* and recalls the somewhat same form and position of *Marattia* described by Jonkman (41). The greater part of the spermatozoid seems to be derived from the nucleus of the spermatocyte. In one or two fortunate sections of spermatozoid I observed the form of the complete spermatozoid (Fig. 8), which is a rather thick, somewhat flattened band consisting of three coils.

ARCHEGONIA.

The archegonia are situated mostly on the lower surface of the prothallium and arise from the cushion region, which is rather massive in this fern. In their earliest stages they are difficult to distinguish from very young antheridia, but very soon their real nature becomes evident. They are in acropetal succession and are more regularly arranged than the antheridia. The mature archegonium consists of a peripheral wall of cells constituting the projecting neck as a cylindrical chimney and a central group arranged serially. Figs. 9 and 10 represent archegonia in median section. The neck projects from the surface of the prothallus, and the projecting portion consists of four rows of cells, each row consisting of about six cells, somewhat flattened and parallel to the surface. The lowest of the three central series is the basal cell, which usually undergoes a few divisions, but its outline remains clearly recognizable in mature archegonia. As the egg-cell proceeds to maturity its nucleus obviously becomes very large and distinct (Fig. 10). Generally one, but sometimes two nucleoli are present in the nucleus, which is not very rich in chromatin. From the arrangement of the cells at the base of the ovum, it seems very likely that a basal cell will be distinguishable in the developing archegonia. The details of the development of the archegonium have not been followed in the few specimens of this age available, but this omission is the less important, since a critical study of the development of archegonia will be communicated in a later paper of this series.

EMBRYO.

The segmentation of ovum has not been traced, but embryos were present on a few of the prothalli. They were mostly of advanced age and presented the appearance represented in Figs. 11, 12, 13. The embryos are conspicuous as a globular projection on the prothallus. The varying positions of the archegonium cause diversity in the direction of the growth in the primary organs of the embryo, and consequently in making sections of embryo considerable difficulty was encountered in orientating the prothalli. The figure drawn was obtained from a comparative study of a large number of serial sections cut in all possible directions, namely, transverse, longitudinal, and horizontal. A median vertical section (Fig. 11) shows the main structural features of the embryo. In form the young embryo is usually globular or somewhat elongated either vertically or horizontally. The succeeding divisions evidently are variable. The number of young embryos available was very small and the common arrangement of the cells in them could not be followed clearly. Bruchmann (8) figures several cases of regular quadrant and octant division for *Botrychium lunaria*, L., but none of the embryos in *Doodia aspera* showed such regularity in the position of the walls of corresponding stages. A median vertical section shows the main structural features. The whole of the lower portion is evidently to be regarded as foot. The smaller upper portion consisted of cells with lot of cell-contents, but the position of the primary organs could not be determined in it. The first wall separating these epibasal and hypobasal halves can be traced, and the quadrant and octant walls also could be followed in each of these halves. After fertilization, the neck of the archegonium generally breaks down. The fertilized egg develops a membrane and the unicellular embryo fills the ventral cavity. It soon divides by a transverse wall and the two cells are almost of approximate size. The facts of segmentation of the fern-embryo, which is the foundation of an elaborate theory of embryology, based upon cell-cleavages, or as Goebel (37) styles it, a sort of 'theory of mosaics', is an extremely interesting but difficult subject, and it is proposed to deal with some of the salient features met with in this material in a later communication. The 'segmental cleavages' in the early states of the embryo play an important part in embryological argument, and this is admirably dealt with by Bower (14) in one of his recent books.

EXPOSITION OF CHROMOSOME CYCLE IN THE PROTHALLIUS.

In order the more clearly to demonstrate the disorganization and reconstruction of chromosomes the more usual plan of beginning the description at the 'resting' stage will be abandoned. It is proposed to begin the description at telophase, for the task of tracing the history of chromatin

through the critical stages—the telophasic transformation of the chromosomes into resting reticulum and the ensuing reconcentration of their elements—is facilitated, and beyond doubt this starting-point is a definite and non-controversial stage.

TELOPHASE.

The chromosomes at very late anaphase or early telophase are grouped at either pole of the fast disappearing spindle (Fig. 14). They begin to adhere to one another at various points and so form anastomoses (Fig. 15). Subsequent stages are rendered somewhat obscure owing to the contracted form of the nucleus and to its densely staining reaction, which obscures detail. As the nuclear wall forms, the chromosomes, after remaining tightly pressed together for a short time, begin to separate from one another (Figs. 15 and 16), and become transformed into a more or less beaded spireme. Some of the anastomoses may originate after the manner of pseudopodia, and others (some early ones) may be formed by the adherence of two or more viscid substances of adjoining chromosomes originally in contact. The former view is upheld by Gates (36), Boveri (5), and by Lundegårdh (49), while the latter view is advocated by Strasburger (84, 85), Müller (59), Déhorne (22). A similar view is held by Sharp for *Vicia* (79) and *Tradescantia* (80). The chromatic granules of the spireme continue to fragment till the nucleus acquires a finely chromatic, granular character. The fragmentation of the chromatic granules was to be seen more especially at the centre of the telophasic chromosomes, which dissolves rapidly, leaving a space bounded on either side by thin threads (Fig. 17, *a*). It is this telophasic vacuolization which Grégoire (39, 40) and others described as skeletons of the former chromosomes. Digby (23), working with *Galtonia*, states that it can be seen, though not so diagrammatically as is described by Grégoire in *Allium*. Sharp (79, 80) lays special emphasis on this vacuolization of chromosomes, which is very prominent in *Vicia* and *Tradescantia*. In fact, whatever it may be, whether vacuolization or alveolization or disintegration of chromosomes giving rise to hollow spaces, it starts at about the time when the chromosomes just begin to separate. The vacuoles, seen first within the chromosomes, are somewhat obscure, but they gradually become more pronounced as circular or oval spaces. The centre of the telophasic chromosomes dissolves, thus leaving a space bounded on either side by thin threads (Fig. 17, *a*). This alveolization of chromosomes in telophase is an important phenomenon, and emphasis must be laid on it since it bears directly upon the question of the interpretation of the splitting of chromosomes. The true telophasic alveolization begins at about the time when the chromosomes start to separate from one another (Figs. 16, 17). The vacuoles, obscure at first, become more pronounced as elongated spaces within the chromosome. They occur only in the median region,

never near or at the periphery, as described by Sharp (79) for *Vicia*. This alveolization, which breaks the chromosomes into two or four, leads to the conclusion that a longitudinal split has occurred, the chromosomes from this stage onward being consequently regarded as double. Attention is therefore directed to Figs. 16, 17, 18, which have been drawn with the greatest care in order to convey as accurately as possible an idea of the real nature and structure of the chromosomes as they appear in the preparations. In Figs. 17 and 18 are shown chromosomes in which there could be no denial of the presence of a split, as it can be seen in section from various angles. No further argument would seem to be required to demonstrate that the chromosomes during these and the later telophase are split threads and so can be called 'double'.

As the chromosomes break up their fragments are seen to flatten out into non-staining portions (linin, Fig. 18, *a*), and also give rise to small rounded particles which may lie parallel to one another (Fig. 18, *b*, *c*). Whatever form and size the linin takes it is seen to stain uniformly. From this, one can arrive at the conclusion that it is impregnated with evenly distributed chromatin. The spindle fibres rapidly give place to a reticulum, and large chromatic bodies are to be seen scattered in the cytoplasm (Fig. 18). The linin breaks up into still smaller pieces, the parallel threads diverge, giving rise to an irregular meshwork. The linin framework is at first more or less uniformly distributed, leaving a clear space round the nucleolus (Fig. 20). The space becomes more pronounced as the nucleus approaches the resting stage.

It is extremely difficult to trace the relationship between the chromosomes of the telophase and the chromatic aggregation of the interkinetal resting stage, because of the apparent dissolution of the chromosomes to form a granular precipitate. Still, a critical examination of the preparations shows without doubt that the chromatic bodies are derived from the preceding mitoses. Davis (19) was able to trace the relationship between chromosomes of the telophase of the last archesporial division and the chromatic bodies of the succeeding heterotype prophase. Nakao (60), on the other hand, does not agree with this, and he is of the opinion that the phase preceding the so-called resting stage exhibits no definite structure.

In the daughter nucleus no continuous spireme is observed. Most previous workers do not report the existence of a continuous spireme at this stage. Merriman (55), however, states that more or less continuous spireme is produced. The nucleolus in my preparations appears during the early telophase. Fraser and Snell (33) state that in *Vicia* it appears as a drop or several drops, usually in relation to the chromosomes. Later stages of the telophase show (Fig. 18) the nucleus to have increased considerably in size, as has also the nucleolus. The chromatic granules of the spireme continue to fragment until the nucleus acquires a fine chromatic granular character.

A comparison of Figs. 15, 16, 17, 18, will aid in interpreting the longitudinal split of the chromosomes. The true relation of the middle alveolization to the splitting of chromosomes will be discussed after the consideration of prophasic phenomena.

INTERPHASE.

In the interphase the telophasic transformation has been carried much farther and the anastomoses cannot be distinguished from the other fine strands of the reticulate chromosomes, and it is extremely difficult to make out the limits of chromosomes. Fig. 19 represents a stage in which the nucleus has increased considerably in size and the whole cavity is filled by a uniform reticulum. It was Lundegårdh (49) who first noticed that in rapidly dividing tissues there is a stage which can be observed between the resting stage and the prophase; to this stage, which is of very short duration, he applied the term 'interphase'. In fact, one cannot say whether a certain nucleus is still undergoing telophasic transformation, or has stopped and entered upon the prophase. In the most active part of the tissues, such as the root meristems or the apical part of the prothallus, some nuclei, without undergoing any further transformation, such as the interphase and rest, may go directly to the prophasic changes of the next division. The anastomoses connecting the reticulate chromosomes begin to break down while the chromosomes are yet distinguishable, so that there is no interval between two successive divisions at which the limits of chromosomes cannot be discerned.

Although in interphase the nucleus grows in size and the cavity is full of a uniform reticulum, still careful observation will lead to the conclusion that the structural identity of the chromosomes is not altogether lost during the short interval between two successive mitoses. Here and there are regions which probably represent boundaries between the constituent chromosomes. One or two nucleoli are invariably present (Figs. 19, 20), and in some nuclei small karyosomes could be observed. The more uniform reticulum with karyosomes may continue in this state for some time, so it may be said to be in the 'resting' state.

RESTING STAGE.

The structure of the reticulum at the resting stage is of the first importance. The contents of the nuclear cavity, at first sight, appear to be nearly uniform though irregular in details (Figs. 21, 22), but careful inspection reveals the fact that along certain lines the strands are rather finer than in the intervening regions (Fig. 23). In fact the linen reticulum is almost colourless, while the chromatin may not be wholly concentrated in the chromatic bodies, but may also be present in the form of numerous small beads

(Figs. 21, 23). The chromatin at first tends to concentrate into small granules, and these gradually collect together in groups to form more definite chromatic aggregations (Figs. 23, 24). The spherical chromatic bodies which are formed in this way take the stain deeply and are very clearly defined from the almost colourless and finely granular reticulum in which they are suspended. In some places the network may show a stranded appearance. A careful examination, together with a comparison of the successive stages (Figs. 21, 23), will show that the heavier bands represent the reticulate chromosomes of the telophase joined together as a continuous net by finer anastomoses. It is not claimed that all nuclei show the individual chromosomes during rest, but what should be emphasized is the fact that the nucleus exhibits certain aspects similar to those present at the telophase (Fig. 23). Thus, one recognizes a considerable number of parallel filaments which might be taken for double bands (Fig. 23) if one had not studied their origin. The chromatic bodies constantly exhibit a paired arrangement (Figs. 21, 23). The origin of this paired arrangement is difficult to determine, because the chromatic bodies are evolved by gradual chromatic concentration. It may be that the pairing is due to the fission of a segment which has condensed as a whole, or it is quite possible that it arises from an early association of separately condensed daughter segments. Even in the same nucleus the upper portion may show the association of chromatic granules which in the lower fission seems to take place.

The present study has revealed the evidence for the pairing of the bands during rest, such as has been reported by Rosenberg (73) for *Drosera*, by Déhorne (22) for *Salamandra* and *Allium*, and by Digby (24) in *Crepis virens*. The evidence obtained in *Doodia* (75) favours the view that the resting reticulum is made up of a linen network upon which the chromatin is borne as individual granules or 'Pangenosomen', as is believed by Merriman (55) for *Allium*. A critical study of variously stained preparations shows that the reticulum is made up of granules of chromatin carried on a supporting network. The structure of the fine reticulum is the direct result of progressive transformations, chromatin granules at resting stage appearing as the denser portions of the alveolated and reticulate chromosome, while the ground network consists of the thinner portion of the same.

From this evidence we must conclude that *Doodia* supports the view of the existence of two principal and morphologically distinct elements in the nuclear reticulum, one, the ground substance, impregnated by the other, the chromatic matter. Such an opinion is held by Grégoire and Wygaerts (38), Grégoire (39), Digby (23), and Lundegårdh (47, 48, 49). Sharp (79) does not agree with this interpretation, as *Vicia* does not afford good evidence for the existence of more than one substance in the chromatic structures. Bonnevie (9, 11) also believes in two substances; she is of opinion that the achromatic material is not continuous from one chromosome

generation to the next. It is interesting to note here that Frisendahl (34) observes in *Myricaria* that in the resting stage, each of the small minute delicate chromosomes is represented by four parts, arising during telophase by a true split and further subdivision.

The study of the nuclei of the resting stage suggests that the chromatic bodies present in the nucleus are merely concentrations or storehouses of chromatin, which substance will subsequently be drawn upon for the evolution of the chromosomes. This agrees with the opinion of Laibach (45), Rosenberg (73), Lundegårdh (47), and Digby (24). It is quite possible that the chromatic bodies may be actual portions of the chromosomes of the preceding mitoses which have remained concentrated during the late telophase.

The threads in most of the resting nucleus are so delicate and rare that the nucleolus appears to lie in the centre of a perfectly free space (Fig. 21). The cytoplasm with fine granules shows a uniform network.

PROPHASE.

The material decidedly suggests that the nuclei pass through an interkinetal rest before entering upon prophase. The passing from the resting stage into very early prophase (Fig. 24) is recognized by the generally more active character of the nucleus, by the sharply staining reticulum, and by the appearance of chromatic staining granules. A decided increase in the size of the nucleus is also obvious, and the meshes of the reticulum have become extended where beads, and fine threads joining the beads together, can be observed. The first indications of approaching mitosis are noticed in the nuclear reticulum (Fig. 24). The chromatin begins to aggregate in small band-like masses and gradually spreads itself and builds up the spireme ribbon. The chromatic bodies which gradually disintegrate, and the substance of which is dispersed throughout the reticulum as chromatic beads, take up a position at the intersecting points of the strands (Fig. 25). The transition stage from 'rest' to the following prophase may be recognized by the gradual disappearance from the cytoplasm of the bright refractive bodies, and by the breaking up of the chromatic bodies into beads. These beads, carried on the linin reticulum, are arranged and grouped in various ways. They may form an irregular cluster (Fig. 25), or they may be arranged serially on an isolated linin strand (Fig. 27), or two such beaded strands may run closely parallel to one another (Fig. 26). From its early stages of formation the spireme is seen to be a double structure. It seems evident that these bands of double structure are the same as are concerned in the make-up of the reticulum at the preceding telophase, since such bands are visible throughout the whole resting stage (Figs. 23, 24). These linin threads, containing the beads of chromatin, tend to run in parallel pairs. The parallel arrangement represents the reassociation of the longitu-

dinal chromosome-halves which had separated during the preceding telophase. The telophasic transformations then produce in the chromosome a real chromatic duality and not merely an *appearance* of duality resulting from an axial alveolization. The separation of the spireme into two parallel longitudinal bands is to be taken as definite fission, and the split first makes its appearance, as will subsequently be shown, in the anaphase of the preceding divisions. The split does not arise at the prophase, as described by Grégoire (39), who is of opinion that the longitudinal division is essentially a prophasic phenomenon; nor is it a phenomenon of metaphase, as stated by Schaffner (76). In almost all the nuclei, even in the early prophase, paired or closely approximated strands can be seen (Figs. 24, 25), indicating the origin of the future chromosomes; each of these strands will become a daughter chromosome at the approaching mitosis. The general tendency of the early prophase is to bring about a gradual concentration of the linin until the completion of the fully formed chromosomes. The early concentration may take various forms. The linin may be condensed into irregular masses (Fig. 28), or into beads (Fig. 29), or into smaller granules (Fig. 30). The more or less isolated granules of the early spireme spread themselves over the nuclear groundwork and they may lie in parallel rows (Fig. 30). These bands and masses may be homogeneous or show alveolization. A careful study of sections shows that there is no regularity in the method of condensation, but the separating portions of the reticulum continue to condense and the anastomoses between them become further broken down, and thus they stand out with great distinctness (Fig. 31). As the early prophasic changes are in many respects the reverse of those occurring in the telophase, the structure of the chromosomes at these two stages is almost alike. It is evident that at this stage of prophase, just as in telophase, each chromosome has an alveolar appearance and in later stages becomes distinctly split (Fig. 32). Some of the vacuoles elongate a little, making the thread clearly double for some distance (Figs. 31, 32). From the structure of chromosomes at this stage it is perfectly clear that the paired beaded threads closely resemble those of telophase—that, in other words, they are retentions from the resting stage and hence from the preceding telophase. These beaded threads tend at first to gather themselves into paired masses; then they gradually disengage themselves and take their place at the points of intersection of the filaments. After condensation of the network, zigzag threads are formed which soon begin to straighten out (Fig. 33), thus giving rise to the definite chromosomes. It is quite possible for the separated portions of the reticulum to be condensed directly into slender threads, representing definite chromosomes, without passing through the zigzag stage. Miss Digby (23) describes a similar occurrence in *Galtonia*, and so does Müller (59), who believes that the threads are formed by direct condensation without passing through the zigzag stage.

The alveolated bands, giving rise to definite chromosomes, are identical with those of the preceding telophase, and after becoming, for the time being, indistinguishable in the network of the resting stage they disengage themselves afresh at the commencement of the division. They thus give rise, by a method of concentration, to chromosomes. Grégoire (39), as the result of careful investigation of *Allium* and certain other Liliaceae, concluded that the 'vacuolization' which occurs in the telophase of a mitosis is responsible for the appearances which have been interpreted by some authors as signifying longitudinal fission. The nearest approach to a representation of such stages is given by Němec (61), who shows in *Allium* the condensation of regions of the net to form irregular vacuolate bands during prophase. The interesting figures of Bonnevie (9, 11) undoubtedly represent schematizations of the zigzag and spiral appearance of the early prophase figures. The early prophase figures of Déhorne (22) seem to have been formed through the application of a similar method to the earlier condensation bands. Yamanouchi (90) found in *Osmunda cinamomea* that the prophaseic bands did not succeed the alveolated bands of the telophase. According to Sharp (79, 80) the chromosomes during telophase become transformed by a process of irregular vacuolization into alveolar-reticulate structures which together make up the resting reticulum, and in the prophase the constituent reticulate chromosomes separate from one another through the breaking down of their connecting anastomoses, each giving rise to a single slender chromatic thread in which the definite split develops as a new formation, though old vacuoles may occasionally be incorporated in it. As a matter of fact, in *Doodia* the figures of resting and early prophase are in complete agreement with those given by Lundegårdh (47, 48) for the plants examined by him. He holds that the resting reticulum gives rise to threads double from the first, the duality probably corresponding to that of telophase. In *Primula*, Digby (26) states that the chromosomes are formed by the stringing together of homogeneous beads of chromatin, a view also in agreement with that of Lundegårdh (47). Beer (13) describes the reticulum as condensing into single or double lines. According to Gates (36) the chromosomes are formed by the fusion of the threads of the reticulum. No evidence is found in my material to support the observation of Merriman (55) that the threads of the early prophase are of a quadripartite nature. The figures show distinctly that the thin threads become increasingly distinct and the chromatic beads are more definitely arranged. Gradually the lengths of threads become closely apposed in pairs and the several paired portions incline to segregate in a form suggestive of the future chromosome.

A short digression must be made here in order to describe other changes which have been taking place in the nucleus during early prophase. During the early stages of prophase the nucleus occupies about half the

cell-volume. The organization of the spireme involves a considerable increase in the size of the nuclear vacuole, so much so that Lawson (42, 43, 44) has described these stages of prophase as the 'growth period'. In the early prophase the nuclear vacuole has a very definite boundary, the nuclear membrane. It is interesting to note, especially in the post-meiotic mitoses of the prothallial cells of *Doodia*, that the growth of the vacuole is accompanied by the stretching or by the growth of its periphery to keep pace with the increased volume. While the stretching of the vacuole is progressing, the chromatic elements become closely pressed to its surface, so that there is very little doubt that this growth is nothing but a result of increased turgidity due to metabolic processes which must be taking place and which result in the formation of the spireme segments. With regard to the form of the vacuole it is not necessarily spherical as described by Lawson (43) in *Allium Cepa*; it may be quite irregular in outline. Farmer (27) has shown that the form of the vacuole will not be determined by osmotic pressure alone, but the surface tension will play a part in the determination of its shape. The increased size of the vacuole is accompanied by a corresponding diminution in definiteness of its boundary. In the older stages of prophase, the nuclear vacuole occupies almost the whole of the cell-space, and one finds very frequently that some of the chromosomes have passed out into the general cytoplasm (Fig. 33). Subsequently, the limits of the vacuole become very faint; there is merely an irregular boundary of cytoplasm (Fig. 35) without the least indication of a membrane with a distinct entity, as suggested by Lawson (42), Němec (61), Reed (70), Merriman (55), and others. Lawson further maintains that when the vacuole has reached its full size it collapses, and that the nuclear membrane becomes wrapped around each chromosome. The bearing of the above conclusions on the synaptic contraction during the heterotype mitoses will be discussed in a later paper.

We may now return to the consideration of the prophases. As the double threads thicken they become throughout their length more uniform in size, and the further concentration of the paired chromatic strands results in the formation of thick, large, and deeply staining chromatic segments (Figs. 33, 34). The halves at this stage lie very close together, and the split is easily seen in almost all parts of the spireme lying in a suitable plane (Fig. 33). In some parts the double nature may be revealed by focusing (Fig. 34). These segments nearly fill the nuclear cavity and gradually lengthen out (Fig. 36), forming a somewhat wavy and discontinuous spireme segment. As the concentration proceeds farther, a somewhat curled spireme results (Fig. 35), the segments of which are seen to straighten out during their final thickening prior to the evolution of the chromosomes. In some of these paired segments the two sides may be still beaded and separate for a distance, then become closely associated to form

a thickened rod (Fig. 35). Gradually in more advanced stages there is an almost complete approximation of the parallel sides. The space between them, when visible, is to be distinguished as the line of fission which will separate the daughter chromosomes on the spindle. As in the early prophase there is a great variety in the character of the linin framework leading to the formation of the chromosomes. In some nuclei the linin is much fragmented (Fig. 33), whilst in others the linin may be in continuous lengths. The separate portions of the fragmented linin are short and rounded (Fig. 34). In either case the linin fragments, from being flat and ribbon-like, become thickened rods (Fig. 38). At this stage the staining capacity increases with the increase of the bulk of these thickened rods. In nuclei at these stages it is quite easy to see several free ends, seen in Fig. 34, which are certainly not due to the microtome knife, as the figure is drawn from a prothallus, mounted whole. Certainly at this time, and in all probability at earlier stages also, the chromosomes form no continuous spireme. During this stage, when the process of condensation is still going on, the fragments gradually retreat from the nuclear cavity (Fig. 36); subsequently the individual fragments unite end to end. When the contraction is at its height the chromosomes are very closely packed and their halves become tightly pressed together, and this concentration of the paired chromatic strands results in the formation of large, thick, deeply staining chromosomes which, as a rule, show only the vestiges of original longitudinal fission (Figs. 37, 38, 39). It is necessary here to emphasize the fact that in post-meiotic nuclei of the prothallus the chromosome fragments are more or less homogeneous and show little sign of longitudinal fission, whereas in the chromosome fragments of the nuclei of the sporophytic apex and roots of this plant the fission is most marked. When the chromosomes are fully formed they lie somewhat polarized towards the nucleolus. The chromosomes thicken and contract and, for the time being, the fission in their substance may be again lost to view (Fig. 39). Then they move away from the nucleolus, and the nuclear cavity enlarges and appears to be propelled to the limit of the nuclear boundary before they are drawn in upon the spindle. The spindle now begins to differentiate in the cytoplasm and the nuclear membrane contracts about the chromosomes. The first signs of the radiations (Fig. 37), which indicate the line of future stress of the fibres, appear when the chromosomes are distributed throughout the nucleus (Fig. 37). In the chromosomes at this stage the split becomes complete, and as they continue to shorten and thicken, their halves become so tightly pressed together that in many preparations they can scarcely be distinguished. The fibres push themselves into the nucleus, and by this time, the nuclear wall having disappeared, the chromosomes collect at the centre of the nucleus. Subsequently, they pass on to the equatorial plate and, after 'loosening up' to an irregular group, rapidly become arranged on the spindle.

METAPHASE.

The chromosomes at the metaphase, at least those passing to the spindle, show every degree of longitudinal fission (Figs. 37, 40, 41). In some the split is merely to be recognized as a shining line in the substance of the chromosomes (Fig. 41), whilst in others it may have so far extended as to separate widely the daughter chromosomes (Fig. 37). In the stages immediately preceding the halves are so tightly pressed together that the split becomes almost invisible and the chromosomes look like homogeneous bodies (Fig. 40). It may be pointed out that this homogeneity is especially characteristic of the chromosomes of the prothallus, because in most of these nuclei the longitudinal fission becomes unrecognizable until the chromosomes are about to separate on the equatorial plate. In striking contrast with the nuclei of the roots, where the longitudinal fission is much more precocious, and may be seen in late spireme, the nuclei of the prothallus shows distinctly that the longitudinal split closes up even during the later stages of prophase.

The transition from late prophase to early metaphase could be followed very clearly in the prothallial cells. While the development of the chromosomes is proceeding, spindle formation takes place. At first, a slight thickening of the cytoplasm around the nuclear membrane breaks down; extremely delicate and fine fibres are visible (Fig. 40) running from one end to another. After comparing the various preparations with different fixatives it was seen that in well-fixed preparations the spindle appears as a very weakly developed structure, and its limits are made out with great difficulty. I have observed in a good many preparations that the poorer the general fixation the more clearly the spindle fibres stand out. Lundegårdh (48), Sharp (79, 80), and others also emphasize this fact. Gates (36) is of opinion that the spindle is a relatively stable structure, a view with which we cannot concur.

When the chromosomes are fully formed they take up their position on the spindle and remain for some time lying against it (Fig. 40). Details of the method of attachment of the chromosomes to the spindle fibres have not been followed, but it is quite evident that they attach themselves to the spindle by one end, and for the time being they may lie at right angles to the plane of the spindle (Fig. 40) and show their nearer extremities, often a little curved (Fig. 40), arranged very regularly in the equatorial plane (Fig. 40). The rest of the body is more or less straight or sometimes curved (Fig. 38). The chromosomes are nearly always disposed according to their length in the equatorial plane, and form a sort of crown which is rather distinct in the metaphase, seen from the pole (Fig. 39). Throughout the development of the spireme the longitudinal fission is more or less clear, but after segmentation it begins to get more and more inconspicuous, and

may sometimes seem to be completely obliterated (Fig. 40). Some of the chromosomes, instead of remaining straight, become somewhat twisted in the form of a more or less wider loop or V (Figs. 39, 40) the sides of which are well extended. The split appears to be more prominent in the straight chromosomes than in the twisted ones. When the daughter chromosomes have almost separated, each pair resembles a widely extended V, or loop, with one longer and one shorter arm, which move towards the poles at anaphase (Fig. 42). The mottled appearance of the chromosomes at this stage is partly due to their uneven contour and partly to unequal density of the chromatin in various portions of the chromosomes.

The nucleolus is pushed off the spindle in the confusion of the chromosome movement. It takes a very deep stain like the chromosomes and is recognized by its globular form. It vanishes with amazing rapidity and leaves practically no evidence of its former existence, even at such an early stage of metaphase, when the chromosomes complete their equatorial arrangement.

ANAPHASE.

The fibres push their way into the nucleus, and the chromosomes collect on the spindle (Fig. 42) just before passing to the poles. The entire univalent chromosomes of each pair move off, one set to either pole (Fig. 43). The two halves of each chromosome begin to separate at the point of insertion on the spindle and gradually move away from the equatorial plane (Fig. 44). A polar view of the equatorial plate shows the great variety in size and shape of the chromosomes (Fig. 45), which is probably due to the different location of the points of fibre attachment and also to the fact that the free ends of the chromosomes occupy various positions. Fig. 43 shows a rather earlier stage of anaphase in which the chromosomes, in the form of loops or rods, are on their way to the poles; the longitudinal fission in some of the chromosomes is still quite evident. In some chromosomes the split is almost through and through, as was seen in metaphase, whilst in others it is visible only in some portions (Fig. 43). As the chromosomes pass to the poles they are generally long and rod-like and slightly hooked (Fig. 44).

The free ends of the chromosomes extend out in various directions, but generally they lie more or less parallel to the axis of the mitotic figure. At this stage the doubleness of the chromosomes reaches its maximum distinctness—a phenomena which is very clearly seen in the somatic chromosomes of *Tradescantia*, studied by Farmer and Shove (31). Just before the daughter chromosomes become completely separated they are seen to take the form of V's, especially when they move at anaphase towards the poles (Fig. 43).

The daughter chromosomes subsequently become entirely free from

one another and gradually draw together into two groups at the poles. Extremely delicate hair-like threads, joining the sister chromosomes, can be observed when the chromosomes are passing to the poles (Fig. 44). The origin of these fine connexions is probably due to the slight projections on the chromosome surface. According to Miss Digby (23) these fine connexions are present throughout the prophases and originally united the linin strands impregnated with chromatin, which will ultimately form the chromosomes. Grégoire (39) also figures and describes these extremely fine connexions. Sharp (79), in *Vicia*, observes that just before the daughter chromosomes become completely separated they are connected by small bridges of chromatic material. Gradually the chromosomes reach the poles and approximate closely to form a confused and more or less homogeneous mass (Fig. 45). At this stage they become much shortened and thickened, and contract into two dense groups in which the limits of the chromosomes can be made out with great difficulty. Still the vestiges of fission can be discerned even at the late stage of anaphase by a shining median line running along each chromosome. Déhorne (22), in his account of *Salamandra* and *Allium*, reports that each chromosome becomes completely split at this time, a phenomena which is difficult to observe in our material. But there is hardly any doubt that the light line which is seen running along the centre of each chromosome is the split, which is gradually disappearing. After a careful observation of a number of slides I have come to the conclusion that this bright line in the centre of each chromosome is not due to phenomena of refraction—a view strongly advocated by Sharp in his paper on *Vicia* (79). It is necessary to mention here some different views with regard to the structure of the chromosome. According to Merriman (55) the chromosomes of *Allium* have at anaphase a tubular structure which soon becomes quadripartite, being truly split. Bonnevie's (9) interpretation is directly opposite to this view: she is of opinion that the cross-section shows at first a tetrad structure which later becomes circular. In her view, the chromatic material is denser at the periphery, so that the axis of the chromosome appears lighter. Fraser and Snell (33) see no recognizable indication of a longitudinal split in the chromosome at this stage, and according to them the chromosome is a single structure. Lundegårdh (48) considered the axial vacuolization in *Allium* and *Vicia* as a true split. Němec (61) also sees, in *Allium*, an axial vacuolization, but does not interpret it as a split.

Fig. 45 shows a later stage where a more compact group of the daughter chromosomes is being formed. Gradually the chromosomes approximate closely to form a confused and more or less homogeneous mass (Fig. 46). In the median plane of the spindle are found indications of a cell-plate between the two groups (Fig. 46), and thus the cycle is completed.

GENERAL CONSIDERATIONS.

It has already been mentioned that the object of the present study of the post-meiotic chromosomes of *Doodia* is to determine more accurately the exact nature of the transformation of chromosomes in the gametophytic tissue and to ascertain to what extent, if at all, this transformation serves to throw any light on the interpretation of the heterotypic chromosomes. Accordingly, I have attempted in the preceding pages to describe fully the series of chromosomal transformations just as they appear. It is now necessary to undertake the critical consideration of the different stages and to discuss the various interpretations put forward by previous workers. It may be said, as a result of a prolonged study of this particular fern, extending over a period of several years, that every precaution has been taken to avoid overlooking important and critical phases of chromosomal evolution.

In the present discussion attention will be limited to the following important points: 1. Presence and significance of the chromatic bodies in the resting nuclei. 2. The individuality of the chromosomes and continuity of the spireme. 3. Splitting of the chromosomes. 4. Method of splitting.

I. *Presence and Significance of Chromatic Bodies.*

The controversial point concerns the definite chromatic bodies found in resting nuclei, and the question arises whether the chromatic bodies are mere aggregations of chromatin or whether they represent prochromosomes.

In this connexion it is necessary that the observations and views of certain investigators should be given very briefly. Roux (74) was the first one who held that the complicated process of mitosis is without any significance unless the chromatin is qualitatively different in different parts of the nucleus, and further that the arrangement of the material of the chromosome in the form of a long thread, just prior to its splitting, is a means whereby all these qualities are equationally divided and distributed to the daughter nuclei. This hypothesis was formulated by the theory of Balbiani (3) and Pfitzner (68), who held that the chromatin granules arrange themselves in a series in the chromosome and by their division initiate its splitting. Later on, Brauer (7) and many other workers put forward the view that the chromatic granules are significant units in the nucleus and that their division is an act of reproduction. Although a good many investigators, especially those interested in the hereditary role of the chromatin, have given special importance to the chromatic granules, others have raised strong objections to the theory that they are significant units or individuals. According to Rosenberg (71), the chromatin bodies agree fairly closely in number with the chromosomes; this Laibach (45) subsequently confirmed, but he did not consider that the chromatic aggregations actually represent chromosomes,

but that they only constitute chromosome centres. Allen (2) has observed the chromatic aggregations in the presynaptic phases of *Lilium canadense* and ascertained that they are more numerous than the chromosomes. Mottier (57) was not able to determine the exact number of the chromatic masses, and moreover found that they varied in size. Strasburger (84, 85) believes that the chromatic granules appear in each successive nuclear division, not only in specific numbers but also in characteristic shapes and sizes. Martens (52) and Davis (19) have found that the chromatic bodies of the resting cell are identical with, or are derived from, the chromosomes of the preceding telophase. A very similar observation has been made by Frisendahl (34), who states that the paired chromatic bodies are transverse sections of the longitudinally split chromosomes of the preceding telophase. A somewhat similar condition is stated by Miss Digby to occur in *Galtonia* (23), though the facts as to the origin of the paired chromatic portions were not so definitely ascertained. Grégoire (38) and Overton (66) have shown that the chromosomes at telophase may resolve themselves into elementary reticula, and that these retain their independence during the resting period and become concentrated again to chromosomes during prophase. Farmer and Moore (30) have observed in *Periplaneta americana* cloudy areas which represent future chromosomes and gradually condense to form them. Miss Digby has also observed complete chromosome disintegration in the resting nuclei of *Primula* (26), and consequently these resting nuclei possess little visible chromatic content. Nakao (60) has figured the resting nuclei as being a little darker than the surrounding cytoplasm and the chromatic bodies gradually making their appearance just before the approach of prophase. Rosenberg (72) and Ostenfeld (65) have shown that the number of chromatic granules forming prochromosomes approximates to that of chromosomes, which is not in accordance with the view of Beer (13). Yamanouchi (89) describes the resting nucleus of the germinating tetraspore as possessing a delicate reticulum dotted with chromatic granules whose number is about the same as that of the chromosomes. Moore and Embleton (58) have observed that chromatic rods are present in the resting nuclei and that their number generally corresponds to that of the chromosomes. Schaffner (76) has shown that the chromatic masses found in the nuclei of *Agave virginica* correspond approximately with the reduced number of chromosomes in prophase. Gates (35) has described chromatic bodies in the nuclei of *Oenothera rubrinervis* which exhibit no constancy in their number or size. Miss Digby's exhaustive study of *Crepis* confirms the view that the chromatic bodies are just the chromatic concentration and not prochromosomes in the real sense of the term. Quite recently Bagchee (16) has made a similar observation on chromatin granules of resting tetrad nuclei. Sharp, in his interesting papers on *Vicia* (79) and *Tradescantia* (80), raised an objection to the theory that the chromatic granules are significant units or individuals.

The observations and views of various authors given above indicate that there is a wide range in the degree of chromosome dissolution at telophase which results in varied concentrations of chromatic elements in the ensuing resting stage. The results of my investigation of the resting nuclei of the prothallus confirm the view that the chromatic bodies are simply the expression of chromatic concentrations. The main argument for this view is that the chromatic granules vary in number and in size. Further, the entire chromatic contents of the nucleus are not always concentrated in these bodies, but the residue may be distributed throughout the reticulum more often at the angles of the meshes. It is true that sometimes the number of the chromatic bodies present in the resting nucleus is the same as that of the chromosomes, but that, in all probability, is a matter of chance. In some cases the identity of the chromosomes remains more or less recognizable, while in others recognition is impossible, but it does not necessarily follow that invisibility involves loss of real identity.

II. *The Individuality of the Chromosomes.*

It is a widely held view that the chromosomes are maintained as autonomous organic units throughout successive generations of cell-division. That they preserve their identity as individuals through the resting phase and interphasic period and maintain a genetic continuity throughout the life-cycle has been contended in numerous publications and is admitted by the great majority of cytologists of whom Van Beneden (4), Rabl (69), and Boveri (5, 6) were the pioneers. On the other hand, a small group, such as Tellyesniczky (86), Nakao (60), Della Valle (20, 21), and Champy (18), hold that the resting nucleus no longer contains real chromosomes, these having undergone at telophase a process of complete dissolution. There would result, according to this view, an interphasic nucleus of homogeneous nature in which the substance of the different chromosomes is mixed. The filamentous aspects, granular or reticular, described and figured at this stage by the authors would therefore be purely artificial in nature. At prophase, chromosomes would be formed in this homogeneous nucleus at the expense of a colloidal material, just as crystals are formed at the expense of a true solution.

This question will not be pursued farther. It appears that the available data on the evolution of chromosomes is only explicable by admitting the morphological persistence of the latter from generation to generation. Certain observations are to be recorded which confirm once more this point of view.

It has been seen, in the interphasic nucleus, that the individual chromosomes, in spite of their apparent dissolution, which renders it difficult to recognize their limits in all cases, do actually continue within the nucleus as

persistent entities. Hence it seems impossible to escape from the conclusion that the chromosomes preserve their autonomy throughout the resting stages. This is especially clear in the post-meiotic mitoses of the prothallus, where the chromatic bodies of the resting stage are identical with, or are certainly derived from, the chromosomes of the preceding telophase. The individuality of the chromosomes can be recognized in the resting nucleus according to the degree of dissolution or dispersion sustained by the substance of the chromosomes of the preceding division. They preserve their identity as individuals throughout the resting phase, and it is evident that the chromosomes to which the reticulum gives rise in the prophase are actually the same as those which went to 'make up' the reticulum at the preceding telophase. In fact in prothallial divisions it was observed that the telophasic dissolution of the chromosomes and their anastomoses to form the reticulum do not proceed so far, during the metaphase, as to obliterate the visible boundaries between them. It is interesting to note here the observations of Marchal (53, 54), who, as a result of studies on the growth stage of animal oocytes, came to the conclusion that the chromosome is not simply a mass of chromatin, but rather 'a structure periodically chromatic'; consequently, the disappearance of stainable matter does not signify the loss of structural continuity.

As a result of an examination of a long series of serial sections, it may be affirmed that the results obtained from this study support very strongly the theory of the individuality of the chromosomes.

Continuity of the spireme. At no stage of telophase or interphase have I observed a union of the chromosomes end to end in such a way as to form a continuous daughter-thread. Thus, I have never found at prophase a continuous spireme which would produce chromosomes by transverse division. From the beginning of prophase just before the spireme stage, one may already observe the numerous free ends which are certainly natural. As development proceeds the spireme is arranged in coils and loops (Fig. 37), radiating somewhat irregularly from the nucleolus in a way that recalls the well-known stage of 'second contraction' of meiosis; this may perhaps be compared to the polarization described by Farmer and Shove (31) in *Tradescantia*.

III. *Longitudinal Splitting of the Chromosomes.*

In order to interpret the critical phenomena of the telophasic splitting it is necessary to follow the sequence of events from the late anaphase, where chromosomes are more or less homogeneous or beaded in nature. Gradually a fragmentation of the chromatin proceeds and the halves separate from each other. The indication of vacuoles can be observed along the axis, which develops into more or less continuous thread. So the definitive splitting of the chromosomes occurs in the telophase rather than the prophase, as several

investigators have urged. The telophasic alveolization is a very regular process, its result being the transformation of each chromosome into parallel threads which may be called double. The nature of the double spireme is to be seen from the earliest stage; at first it consists of a double series of granules which place themselves on a nuclear framework, side by side. The dissociation or separation of the spireme into two distinct longitudinal bands is regarded as evidence of a definite fission. Sharp (79), in describing the somatic chromosomes in *Vicia*, is of opinion that the longitudinal splitting is a phenomenon of prophase. He showed that at telophase the chromosome becomes transformed into irregular reticulate bands which remain connected as a continuous network through interphase or more complete rest, and which again separate from each other at prophase. Each then condenses in an irregular manner and gives rise to a thin zigzag thread. After this thread becomes regular the longitudinal split occurs as a result of median vacuolization, the split so formed remaining until the separation of daughter chromosomes at metaphase. Grégoire (40) is also of opinion that longitudinal division is essentially a prophasic phenomena. Although he and other investigators agree in ascribing the process to a fairly early stage, they nevertheless differ concerning the precise moment when the splitting is produced. On the one hand, according to several authors, division is only effected at the moment when the chromosomes are inserted on spindle fibres. On the other hand, there is the widely shared opinion that integral bipartition of the elements of chromosomes is produced by means of a series of vacuoles formed along the axis. These vacuoles accordingly would unite to form a continuous split separating the sister halves of the same chromosome.

The evidence afforded by *Doodia* leads to the conclusion that the telophasic vacuolization is to be regarded as a splitting or, more precisely, that the chromosomes of the late telophase, resting stage, and early prophase should be regarded as already double. Chromosomes in this condition are double in the real sense of the word. They contain open spaces which later join with others to form a split. As a result of careful observation of the telophasic and prophasic changes, and an examination of a large number of serial sections, the writer contends that the definitive splitting of the chromosome occurs in telophase rather than in the prophase. That such a state of things is of common occurrence is suggested by the observations of Grégoire and Wygaerts (38) on *Trillium*, Digby (28) on *Galtonia*, and Fraser and Snell (33) on *Vicia*. The recognition of the longitudinal fission in the chromosome is thus carried back from the prophase to the preceding telophase. Recently, Newton (63), in his interesting study on the somatic chromosomes of *Galtonia*, has shown that the telophasic vacuolization is sufficiently regular in *Galtonia* to afford support to the theory that it involves the actual longitudinal splitting of the chromosomes. He further states that

in order to secure definite proof it should be possible to demonstrate continuity between the telophasic vacuolization and the definitive split of prophase; this, however, has not been found easy in these plants. More recently, however, Martens (51), in his study of the cycle of the somatic chromosomes of *Listera ovata*, states that the chromatin bipartition is neither the result of an alveolization of the filament nor of the individual partition of the chromatic particles linearly disposed. According to him the chromatin elements display their filamentous nature in very early stages of prophase. They are neither plates of chromatin nor walls of vacuoles or alveoli as in telophase.

The results here put forward seem to show clearly that the definitive splitting of the chromosomes occurs in the telophase and not in the prophase, and that the telophasic vacuolization is such a regular process that one can even trace the duality—which is the direct outcome of vacuolization—throughout the late telophase, resting stage, and early prophase. Such being the case the chromosomes may be said to be double in the real sense of the word.

IV. *Method of Chromosome Splitting.*

It is rather difficult to obtain convincing evidence as to the exact manner in which the separation of the daughter chromosomes takes place, but the stages of separation are comparatively easy to follow in the post-meiotic mitoses of the prothallus. The chromosomes become attached by one end to the spindle and for a time they remain so, but subsequently are swung out at right angles to the axis of the spindle and then separation of the two halves begins at the attached end. The halves remain in contact at the free end for the time being, and thus the two daughter chromosomes form first an acute angle and then an obtuse angle, till they are almost in a straight line; by this time they are quite free from one another. The exact manner of chromosome splitting has a direct bearing on the problem of the mechanism of heredity. The view that the splitting of the chromosome is essentially a division of a series of smaller units which it contains has been widely accepted, especially by those who are interested in the cytological aspects of inheritance.

While the development of the chromosomes is proceeding, spindle formation takes place. It is a well-known fact that in well-fixed preparations the spindle appears as a very weakly developed structure and its limits are to be made out with great difficulty. In this connexion it might be said that Farmer and Moore (30) have regarded them as protoplasm modified by electrical forces at work in the cell, to which Fraser and Snell (33) add a suggestion that an important part may be played by currents of altered cytoplasm. In the somatic cells of *Tradescantia*, as in *Vicia*, Sharp has observed that chromosome splitting seems to be initiated by a series of

axial vacuoles, and not by the division of the chromatic granules contained in the thread.

It appears in *Doodia* that the fibres push through the cytoplasm and the chromosomes collect on the spindle just before they pass to the poles. The univalent chromosomes of each pair move off, one to either pole, and thus the cycle is completed.

SUMMARY AND CONCLUSIONS.

1. In *Doodia* the chromosomes in the post-meiotic divisions of the prothallus correspond exactly with those of the somatic and premiotic divisions. They are formed from the telophase of the preceding division by a process of alveolization and partial separation of the two parts, which subsequently undergo a process of reapproximation.

2. The chromosomes preserve their identity as individuals through the resting phase and interphase, and maintain a genetic continuity throughout the life-cycle. Definite evidence has been found in the prothallial divisions that the chromosomes to which the reticulum gives rise in the prophase are actually the same as those which went to the 'make-up' of the reticulum at the preceding telophase.

3. The definitive splitting of the chromosomes occurs in the telophase and not in the prophase, for the telophasic alveolization is sufficiently regular to allow of the duality being traced throughout the late telophase, resting stage, and early prophase.

4. The prothallus of *Doodia* affords trustworthy evidence of the permanence of the chromosomes in the state of autonomous, organic units during the interphasic period, and so supports the theory of the individuality of the chromosomes.

5. There is no continuous single spireme thread.

6. The phenomena in connexion with the telophase of one division have an important bearing on the interpretation of the phenomena found in the early prophase of the next division.

7. The telophasic transformations produce in the chromosome a real chromatic duality and not a mere appearance of duality as a result of axial alveolization, as some workers have held.

In conclusion, I should like to take this opportunity of acknowledging my indebtedness to Professor J. Bretland Farmer, for generous and helpful advice, for guidance and criticism, and also for the many facilities he has so kindly afforded me during the conduct of this investigation. I wish to record my high appreciation of the kindness of Miss Lettice Digby in placing her excellent preparations at my disposal, and for her very valuable advice and helpful criticism throughout the investigation. My sincere thanks are

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EXPLANATION OF PLATES I-V.

Illustrating Dr. Sarbadhikari's paper on the Cytology of *Osmunda* and *Doodia*.

All the figures were drawn with the aid of an Abbe camera lucida under a lens 2 mm. apochr. N.A. 1.4 and comp. ocular 18 at a magnification of about 2,250, except Figs. 1, 2, 11, 12, which were drawn with Leitz 1/12 A immersion and ocular 8 at a magnification of about 1,090.

PLATE I.

Fig. 1. Apex of a young prothallium of *Doodia aspera*, R.Br., showing stages of vegetative divisions.

Fig. 2. Prothallus as seen from below, bearing sexual organs among its rhizoids.

Fig. 3. Part of a transverse section of a tissue near the apical indentation, showing most of the cells in the resting stage. Note the enormous size of the nucleus in some of the cells.

Fig. 4. The same stage as Fig. 3. The pairing of the chromatin granules may be observed in one of the cells.

Fig. 5. Cross-section of a young antheridium, showing sperm-cells.

Fig. 6. A magnified drawing of a transverse section of a ripe antheridium with cuticle partly ruptured. The very numerous spermatocytes with cilia are in division. Note the various forms of spermatocytes.

Fig. 7. Cross-section of a fully matured spermatozoid.

Fig. 8. The same in advanced stage. The spermatozoid shows distinctly about three complete coils.

Fig. 9. Young archegonia.

Fig. 10. Transverse section of a mature archegonium.

Fig. 11. Vertical section of an embryo. *e.*, embryo ; *p.*, prothallus.

Fig. 12. Longitudinal section of an embryo, showing division figures.

Fig. 13. The same in high power.

Fig. 14. Very late anaphase in which the chromosomes are fused into masses. The light line which is seen running along the centre of some of the chromosomes is the vestige of split.

PLATE II.

Fig. 15. Early telophase. As the nuclear wall forms, the chromosomes, after remaining tightly pressed together for a short time, begin to separate one from another, and become transformed into a more or less beaded spireme.

Fig. 16. Telophase, showing the alveolization of the chromosomes and the consequent parallelism of two halves.

Fig. 17. Late telophase, showing also the vacuolization of chromosomes. The central portion is seen to be dissolving, leaving the peripheral portions as parallel threads with somewhat beaded appearance. *a* shows the parallel threads with a somewhat beaded appearance.

Fig. 18. Still more advanced stage of telophase. The alveolated portions of chromosomes are breaking up (*a*, *b*, *c*) into parallel rows of granules, showing the parallel arrangement of the remains of some of the chromosome bands. The linin breaks up into small rounded particles which lie parallel to one another.

Fig. 19. Interphase. The nuclear cavity is full of uniform reticulum.

Fig. 20. The more uniform reticulum, showing that structural identity of the chromosomes is not altogether lost during this short interval between two successive mitoses.

Fig. 21. Resting stage. The chromatin tends to concentrate into small granules, and these gradually collect together in groups to form more definite chromatic aggregations. There is a clear space round the nucleolus.

Fig. 22. Resting stage, with linin network.

Fig. 23. Very early prophase, showing the parallel rows of linin granules.

Fig. 24. Early prophase. The parallel rows of linin granules condensing to form concentrated portions of chromosome bands.

Fig. 25. Still further concentration of the paired linin strands. Note the three nucleoli from which some of the linin threads emerge.

Fig. 26. The same as in Fig. 25. Further concentration of the paired linin threads.

Fig. 27. Slightly later stage in the concentration of the strands. Note the alveolization in the strands, and the longitudinal fission which results from the development of a space between the condensing strands.

PLATE III.

Fig. 28. Nucleus, showing uneven concentration of the linin.

Fig. 29. The chromatin from the beads gradually infiltrating the linin threads. Note the empty space round the nucleolus.

Fig. 30. The beaded linin threads show a striking parallel arrangement. Note the exact similarity of the approaching sides.

Fig. 31. Nucleus, showing the close association of the two threads; the resulting filament becomes considerably thickened.

Fig. 32. Late prophase, showing spireme segmented into chromosomes with longitudinal fission very well marked.

Fig. 33. The chromosomes are fragmenting and thickening. Note the longitudinal fission.

Figs. 34, 35. Longitudinally split chromosomes collecting in the centre of the nucleus.

Fig. 36. The spireme shows a very striking pairing along its length.

Fig. 37. Spindle fibres making their appearance in the cytoplasm.

Figs. 38, 39. The chromosomes are concentrating rapidly at the equatorial plate.

Fig. 40. The spindle fibres invade the nucleus and the chromosomes show a considerable concentration of their material.

PLATE IV.

Fig. 41. The limiting nucleus membrane has disappeared, the chromosomes concentrate, and vestiges of fission are to be seen.

Fig. 42. Equatorial plate stage showing the V-shaped chromosomes about to separate from each other.

Figs. 43, 44. Anaphase. The daughter chromosomes show no fission as they proceed to their respective poles.

Fig. 45. Later anaphase. The chromosomes approximate closely to form a confused and more or less homogeneous mass.

Fig. 46. Very late anaphase in which the chromosomes are fused into masses in the middle of which vacuoles are formed. A cell-plate is to be seen between the two nuclei.

Studies in the Cytology of the Anacrogynae.

III. Fertilization in *Fossombronina angulosa*.

BY

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With Plates V and VI and four Figures in the Text.

CULTURES of female plants of *Fossombronina angulosa*, Raddi, of unknown origin were given to me by the Chef de Cultures of the Jardin Botanique de l'État, Brussels. Plants of the two sexes were collected in November, 1922, near Harlech, Wales, by Mr. D. A. Jones. No differences between the plants from the two sources were noted, and, for the study of the normal fertilization, female plants from the former source were inseminated with antherozoids of plants from the latter source. Plants from both these sources were sent to Columbia University, where cultures were prepared and cared for under the supervision of Professors C. E. Allen and R. A. Harper, to whom my thanks are due. These cultures were later transferred to one of the greenhouses of the University of Wisconsin, where the attempts were made to hybridize *Fossombronina* with *Sphaerocarpos* and with *Funaria*.

The same technique of insemination described in the preceding paper of this series was used in the present case. Material for cytological study was fixed at intervals after insemination, using the fixative described in the preceding paper with the higher concentration of acetic acid. Good fixation was obtained also with the lower concentration of acetic acid and with Tellyesniczky's acetic acid-bichromate solution diluted with an equal volume of water. The air-pump was used to remove bubbles of air which otherwise remain between the lobes of the thallus and interfere with the penetration of the fixing solution.

The washing, dehydration, and embedding were done in the manner previously described for *Riccardia*, except that a longer time was found necessary for infiltration by paraffin.

Serial sections were cut at a thickness of 10μ and stained with the triple stain. The iron-haematoxylin stain succeeds fairly well after the

acetic acid-bichromate fixation, but is entirely unsatisfactory following fixation in the chromic-osmic-acetic acid solution. Sections unbleached and unstained are useful for studies of the plastids.

For the study of the normal fertilization three series of fixations were made after inseminations on Oct. 22, 1923, at 10.45 a.m., and on Oct. 26, 1923, at 10.10 a.m. and at 5.40 p.m. The temperature in the greenhouse in Brussels at this season varied from 9° to 14° C., averaging about 11°. One culture of female plants of *F. angulosa* was inseminated on Oct. 30, 1923, at 3.35 p.m., with antherozoids of *Riccardia pinguis*, and a series of fixations was made extending up to ninety hours after insemination. Insemination of female plants of *F. angulosa* with antherozoids of *Sphaerocarpos Donnellii* was made on Nov. 29, 1924, at 10.45 a.m., in the greenhouse at the University of Wisconsin, the temperature being fairly constant at 15° C., and fixations were made up to 166 hours thereafter; that with antherozoids of *Funaria hygrometrica* was made on Feb. 23, 1925, at 4.10 p.m., temperature 15° C., and fixations extended up to twelve days after insemination.

FERTILIZATION BY *F. ANGULOSA*.

Structure of the Archegonium and Egg.

The thallus of *Fossombronia* consists of a stem-like midrib and leaf-like lateral lobes. Numerous long rhizoids project downward from the midrib and attach the plant firmly to the soil. The sexual organs are produced in acropetal succession on the dorsal surface of the midrib. Sexual organs may be found at almost any season of the year, but are most numerous during the autumn and early winter.

Mature archegonia are found on the female plant a short distance behind the apical region, and archegonia in various stages of development nearer the apical cell. The archegonia are not surrounded by special scales or papillae, but are sometimes covered by the overlapping lobes of the thallus. Each archegonium consists of a distinct venter and a long, slender neck. The wall of the venter is two cell layers in thickness; that of the neck has only one layer. The neck is usually curved sharply, and longitudinal sections are obtained only rarely. The neck canal is narrow in comparison to that of *Riccardia*, and frequently contains clumps of amorphous darkly staining material.

The egg is nearly spherical and about 25 μ in diameter. It does not nearly fill the cavity of the venter in which it lies. The cytoplasm is dense, coarsely granular, and stains deeply. A delicate plasma membrane forms a relatively smooth surface. Humphrey (1906)¹ reports 'a well-defined receptive spot' in the egg of *F. longiseta*, but I find none in this species. The nucleus

¹ The references given in this paper refer to the literature cited in the *second* paper (this Journal, vol. xl, pp. 713-26, 1926) of this series.

is spherical, about 10μ in diameter, and lies near the centre of the egg. It contains one large nucleole about which the chromatin is aggregated in a dense mass (Pl. V, Fig. 1). In no case has more than one nucleole been found in the nucleus of an unfertilized egg. Plastids are generally not definitely recognizable in bleached and stained sections of the egg. Unbleached and unstained sections of material killed in the solution containing osmic acid show in each egg several bodies having the appearance of elaioplasts.

The egg apparently remains capable of fertilization for a considerable length of time. Female thalli fixed after one insemination were found each to bear from several to twelve (occasionally more) fertilized eggs. Plants inseminated with antherozoids of *Funaria*, which penetrate the eggs of *Fossombronia* but do not initiate development, were fixed twelve days after insemination. These plants show eggs containing the foreign antherozoids, and so well preserved that they cannot be distinguished from eggs recently inseminated.

Penetration of the Antherozoid into the Egg.

The penetration of the antherozoid into the egg seems to be instantaneous, and is accompanied by a swelling of the antherozoid. Seven thalli fixed six minutes after insemination show thirty-five eggs already penetrated by antherozoids. The majority of these eggs contain each more than one antherozoid (or male nucleus). A few apparently functional eggs contain no antherozoids. Only one of the thirty-five cases shows an additional antherozoid in the venter outside the egg.

At this stage the antherozoid (or male nucleus) lies in the outer part of the cytoplasm of the egg (Pl. V, Fig. 1). It is considerably shorter than the body of the free-swimming antherozoid, and several times as thick. A hyaline zone whose boundaries are not clearly defined surrounds the male nucleus. The egg figured contains two male nuclei and two pairs of thin fibres are present in the cytoplasm of the egg. These may or may not be the cilia of the two antherozoids which have entered the egg (Pl. V, Fig. 1).

Changes preceding the First Division.

No significant change in the structure of the egg is perceptible during the first thirty hours after fertilization (Pl. V, Fig. 2). Eggs fixed from forty-two to fifty hours after insemination, however, show considerable change. The size of the egg has increased noticeably, and its cytoplasm has become vacuolate, staining much less deeply (Pl. V, Figs. 3, 9). In some cases the nucleus also has undergone significant changes. The chromatic mass about the nucleole has become a reticulum which now occupies approximately one hemisphere of the nuclear cavity (Pl. V,

Fig. 4, lower half, Figs. 5-7 right, Figs. 9, 10 upper focus). In the other hemisphere is a dense mass of chromatic substance which, with the triple stain, is more red than the (maternal) chromatic reticulum (Pl. V, Figs. 4-7, 9, 10). The actual penetration of the male nucleus into the female nucleus has not been observed, but it seems highly probable that this dense chromatic mass is the substance of the male nucleus. In one case the form of this mass is much like that of a male nucleus before its passage into the female (Pl. V, Figs. 3, 4).

Pl. V, Figs. 4 to 7 represent the condition of nuclei of zygotes fixed forty-two hours after fertilization from material inseminated at 5.40 p.m. The greater part of the zygotes found in the material of the same fixation show nuclei only slightly changed from the condition characteristic of the nucleus of the unfertilized egg, and it may be inferred that, in these cases, no nuclear union has taken place (Pl. V, Fig. 8). Figs. 9 and 10 represent zygotes 45 $\frac{3}{4}$ hours after fertilization in plants inseminated at 10.45 a.m. In all these cases one or more supernumerary male nuclei are present in the cytoplasm of the egg, but no evidence has been found that more than one male nucleus has in any case entered the same female nucleus. The supernumerary male nuclei retain their form, but gradually lose their staining capacity until they are no longer visible. They are sometimes recognizable as late as the stage of the two-celled embryo (Pl. VI, Fig. 24). In this species polyspermy appears to be the rule rather than the exception, but the supernumerary male nuclei do not interfere with the normal development of the zygote. Rickett (1923) finds in *Sphaerocarpos* that polyspermy results in degeneration of the zygote.

The mass of paternal chromatin soon becomes optically heterogeneous, and one or more nucleoles appear in it (Pl. V, Figs. 7, 11). It too becomes evenly distributed and forms a reticulum which is continuous with and indistinguishable from that of the maternal chromatin (Pl. V, Figs. 12, 13, 15, 16). Except in rare cases (Pl. V, Fig. 17), the number of nucleoles in the fusion nucleus is reduced to one before the prophase of the first division (Pl. V, Figs. 13, 15, 16).

The First Division in the Zygote.

The first sporophytic division occurs from six to nine days after fertilization, and is essentially like that described in *Riccardia*. The zygote at the time of this division has attained a volume several times that of the unfertilized egg. It is ovoid in shape, the end towards the neck of the archegonium being the larger. The nucleus occupies a position slightly displaced from the centre towards the smaller basal end (Pl. V, Figs. 14, 18).

The transformation of the chromatic reticulum into smooth, rod-shaped

chromosomes is accompanied, as in *Riccardia*, by a migration of the granular cytoplasm to form two aggregations near the nucleus about the positions later occupied by the poles of the spindle. During these changes in the nucleus the maternal and paternal chromatin are indistinguishable, as are also the maternal and paternal chromosomes in the later prophases (Pl. V, Figs. 15–17, 19). No continuous spireme has been observed. The chromosomes are fairly evenly distributed in the nuclear cavity, and appear to be sixteen in number (Pl. V, Fig. 19). The chromosomes in the gametophytic nuclei have not been studied critically, but the number most frequently counted in the female thallus is eight. The nucleus becomes elongate in the direction of the long axis of the zygote, its ends projecting into the aggregations of granular cytoplasm (Pl. V, Figs. 14–19).

During the early prophases the two aggregations of granular cytoplasm show each a radiate configuration (Pl. V, Figs. 14, 18). The disappearance of the nuclear membrane seems to be abrupt and simultaneous with an equally abrupt appearance of the achromatic spindle (Pl. V, Figs. 18–20). The radiate configuration of the granular cytoplasm disappears apparently simultaneously with these changes. The chromosomes appear to migrate to the equatorial region of the spindle very shortly after the disappearance of the nuclear membrane and the appearance of the spindle. I have found no stage intermediate between that of the nucleus with membrane intact, and that of the fully formed spindle with chromosomes in the equatorial region (Pl. V, Fig. 20). The spindle is rather slender and its two poles are quite sharp.

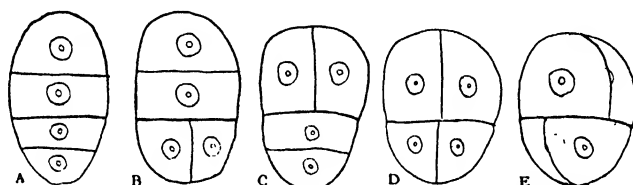
The daughter chromosomes separate and pass to the poles (Pl. VI, Fig. 21). The cell-plate begins to form in the centre of the spindle and extends outward, following the addition of new strands to the spindle, until it reaches the surface of the zygote (Pl. VI, Figs. 22, 23). The strands of the spindle, especially those formed after the separation of the daughter chromosomes, appear to be granular rather than fibrous.

Early Embryogeny.

The first segmentation of the zygote results in the formation of two cells of unequal size, a larger epibasal cell and a smaller hypobasal cell (Pl. VI, Figs. 23–5). The epibasal cell grows more rapidly than its sister and contains larger and more numerous vacuoles in its cytoplasm. The second division occurs almost simultaneously in both daughter-cells, and no three-celled embryo has been observed. A few cases have been found showing the second nuclear division in progress. In these cases the stage of division is slightly more advanced in the hypobasal than in the epibasal cell (Pl. VI, Fig. 26). The planes of this division in the two cells are very variable. Text-fig. 1 represents diagrammatically five different types of

four-celled embryos observed. In some cases the second division is transverse in both of the two cells (Text-fig. 1, A). More frequently it is longitudinal in the hypobasal cell and transverse in the epibasal one (B). Occasionally it is transverse in the hypobasal and longitudinal in the epibasal cell (C). Rarely it is longitudinal in both (D), and a single case has been found in which the three dividing walls (that of the first division and the two of the second) are nearly perpendicular each to the other two (E). Embryos of two or more of these types may be found developing on the same thallus.

It frequently happens, especially when a large number of eggs on the same thallus are fertilized, that some of the resultant zygotes do not undergo any of the changes normally accompanying development. These zygotes may be found on plants fixed ten days after fertilization, and in



TEXT-FIG. 1. Diagrams of the different types of embryos at the four-celled stage.

appearance are indistinguishable from recently fertilized eggs. I have, however, found as many as eight apparently normal two-celled embryos on the same thallus. Female plants left in the culture after a single insemination frequently bear from four to eight mature sporophytes.

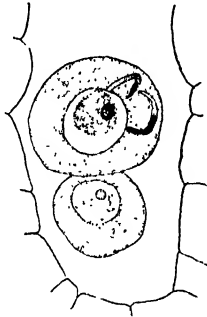
Aberrant Archegonia.

Aberrant archegonia seem to be much less frequent in this species than in *Riccardia pinguis*. The ventral canal cell sometimes persists as a small spherical cell with dense, granular cytoplasm, and a small nucleus. One case has been found in which what seems from its position to be the ventral canal cell has the appearance of a normal egg and has been fertilized (Text-fig. 2). In this case the egg is small and has not been fertilized.

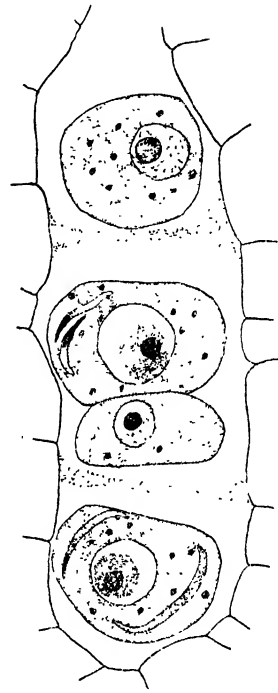
In another case four egg-like cells appear in a row. Two of these show the chromatin of the nucleus aggregated about the nucleole, and male nuclei are present in the cytoplasm (Text-fig. 3). This is in a plant fixed 168 hours after insemination, but there is no evidence of post-fertilization development in either of the cells containing male nuclei.

Another case, in a plant fixed 100 hours after insemination, shows twin zygotes apparently developing normally (Text-fig. 4). The chromatic substance of each of the nuclei is evenly distributed, and has the reticulate arrangement characteristic of the zygote nucleus at this stage of develop-

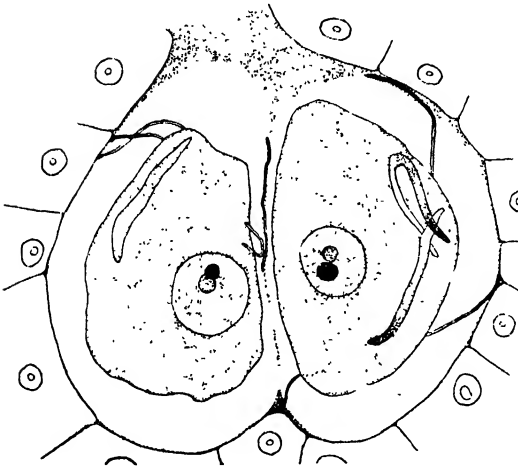
ment. Each nucleus contains two nucleoles, and the cytoplasm is vacuolate. A large amount of deeply staining amorphous substance is present in the neck of the archegonium and extends into the venter. Fixation was attended in this case by an unusual degree of shrinkage of the zygotes.



TEXT-FIG. 2. Aberrant egg and ventral canal cell, the latter of which has been fertilized, 2 hrs. 8 min. after insemination. $\times 1,050$.



TEXT-FIG. 3. Four egg-like cells in an aberrant archegonium. Two of them have received antherozoids, but show no evidence of development, 168 hrs. after insemination. $\times 1,050$.



TEXT-FIG. 4. Twin zygotes, apparently developing normally, 100 hrs. after insemination. $\times 1,135$.

This is one of the rare cases in which supernumerary antherozoids are found in the venter of the archegonium as well as in the cytoplasm of the developing zygote or zygotes.

FERTILIZATION BY ANTHEROZOIDS OF OTHER SPECIES.

One culture of female plants of *F. angulosa* was inseminated with antherozoids of *Riccardia pinguis*, and a series of fixations was made extending to ninety hours after insemination. The fixed material shows that the eggs have been penetrated by these foreign antherozoids, but no

indication of development is perceptible (Pl. VI, Fig. 27). In most cases more than one antherozoid has entered the egg and lies in the cytoplasm surrounded by a clearer zone. The antherozoid (or male nucleus) of *Riccardia* has a different form in the egg of *Fossombronia* from that which it displays in the egg of *Riccardia*, being somewhat shorter and much thicker (compare Pl. VI, Fig. 27, with Pl. XXV, Figs. 1-6, of the second paper (loc. cit.) of this series). No embryos appeared in the plants left growing in this culture.

Female plants of *F. angulosa* were inseminated Nov. 29, 1924, with antherozoids of *Sphaerocarpos Donnellii*, and a series of fixations was made extending to 166 hours after insemination. Plants fixed five minutes after insemination show (*Sphaerocarpos*) male nuclei in nearly all the mature eggs (Pl. VI, Fig. 28). In nearly all these eggs there are a considerable number of male nuclei, some of which lie close together or overlap one another, so that their form or number cannot be determined accurately. The egg represented in Pl. VI, Fig. 28, is unusually large, but was figured because it shows most clearly the form of the male nuclei in its cytoplasm. Plants fixed two days or longer after insemination show fertilized eggs, the cytoplasm of which appears to have undergone some of the changes characteristic of the early development of the normal zygote in this species. The volume of the egg has increased noticeably, and its cytoplasm has become vacuolate and light-staining (Pl. VI, Fig. 30). The egg nucleus in nearly all these cases shows no significant change from its condition in the unfertilized egg. I have found, however, two cases (fixed 96 and 120 hours respectively after insemination) in which the egg nucleus appears very similar to that of the normal zygote of the same age (Pl. VI, Fig. 29). The (*Sphaerocarpos*) male nuclei in the cytoplasm of the egg of *Fossombronia* after four days retain their form, but stain very lightly, and in some cases are difficult to recognize (Pl. VI, Figs. 29, 30). It is perhaps worthy of note that the male nucleus of *Sphaerocarpos* in the egg of *Fossombronia* does not assume the 'resting' condition as it does in the egg of the same species (Rickett, 1923).

Female plants of *F. angulosa* were inseminated Feb. 23, 1925, with antherozoids of *Funaria hygrometrica*,¹ and fixations were made up to twelve days after insemination. Plants fixed six minutes after this insemination show dark-staining, greatly swollen male nuclei in the cytoplasm of the eggs (Pl. VI, Fig. 32). Pl. VI, Fig. 31 represents the antherozoid of *F. hygrometrica* drawn at the same magnification as Pl. VI, Fig. 32. No evidence of development is perceptible in any of the eggs of *Fossombronia* penetrated by antherozoids of *Funaria*. Eggs fixed after twelve days (Pl. VI, Fig. 33) are indistinguishable by their appearance from those fixed after six minutes.

¹ Dr. A. J. Grout has kindly confirmed the identification of this moss.

SUMMARY.

1. The antherozoid of *Fossombronina angulosa* penetrates the egg of this species instantaneously, and usually more than one antherozoid enters the same egg.

2. The fertilized egg shows no significant change for about thirty hours after the antherozoid has entered its cytoplasm.

3. Zygotes fixed forty-two to sixty hours after insemination sometimes show the paternal chromatin distinguishable from the maternal in the fusion nucleus, but the actual passage of the male nucleus into the female nucleus has not been observed.

4. The zygote increases greatly in size, and undergoes the first division from six to nine days after fertilization.

5. The form of the young embryo varies greatly.

6. The eggs of *Fossombronina* are penetrated by antherozoids of *Riccardia*, *Sphaerocarpos*, and *Funaria*.

DEPARTMENT OF BOTANY,
UNIVERSITY OF WISCONSIN,
April, 1925.

ADDENDUM.

While this paper was in the press female plants of *F. angulosa* were inseminated with antherozoids of two additional species—*Fossombronina longiseta* and *Asterella Californica*. Cytological studies showed that these antherozoids also enter the eggs of *F. angulosa*, but no evidence of development was found. These experiments, as in the previous cases of insemination with foreign antherozoids, were not controlled by simultaneous inseminations with antherozoids of the same species. It is, therefore, not certain that in any of these cases the eggs which have received foreign antherozoids would have developed if they had received antherozoids of the same species. Nor is it certain that development of the eggs of this species may not under other conditions be initiated by foreign antherozoids. Female plants growing in contact with male plants of the same species do not always produce sporophytes, although reproductive organs of both the sexes may be abundant.

EXPLANATION OF PLATES V AND VI.

Illustrating Dr. Showalter's paper on Fertilization in *Fossombronina angulosa*.

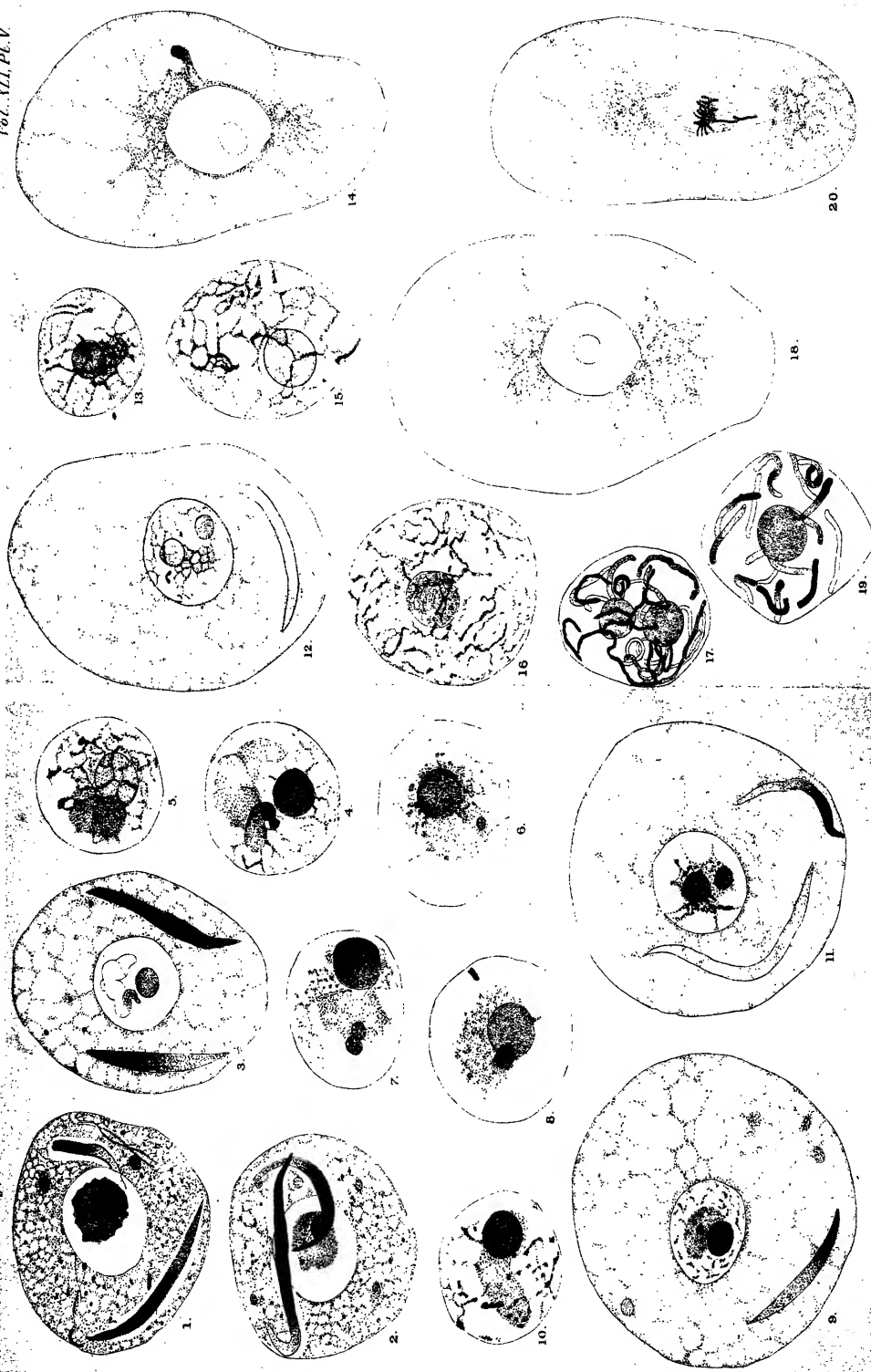
All figures were drawn from stained preparations with the aid of the Abbe camera lucida at the magnification indicated, and are reproduced without reduction.

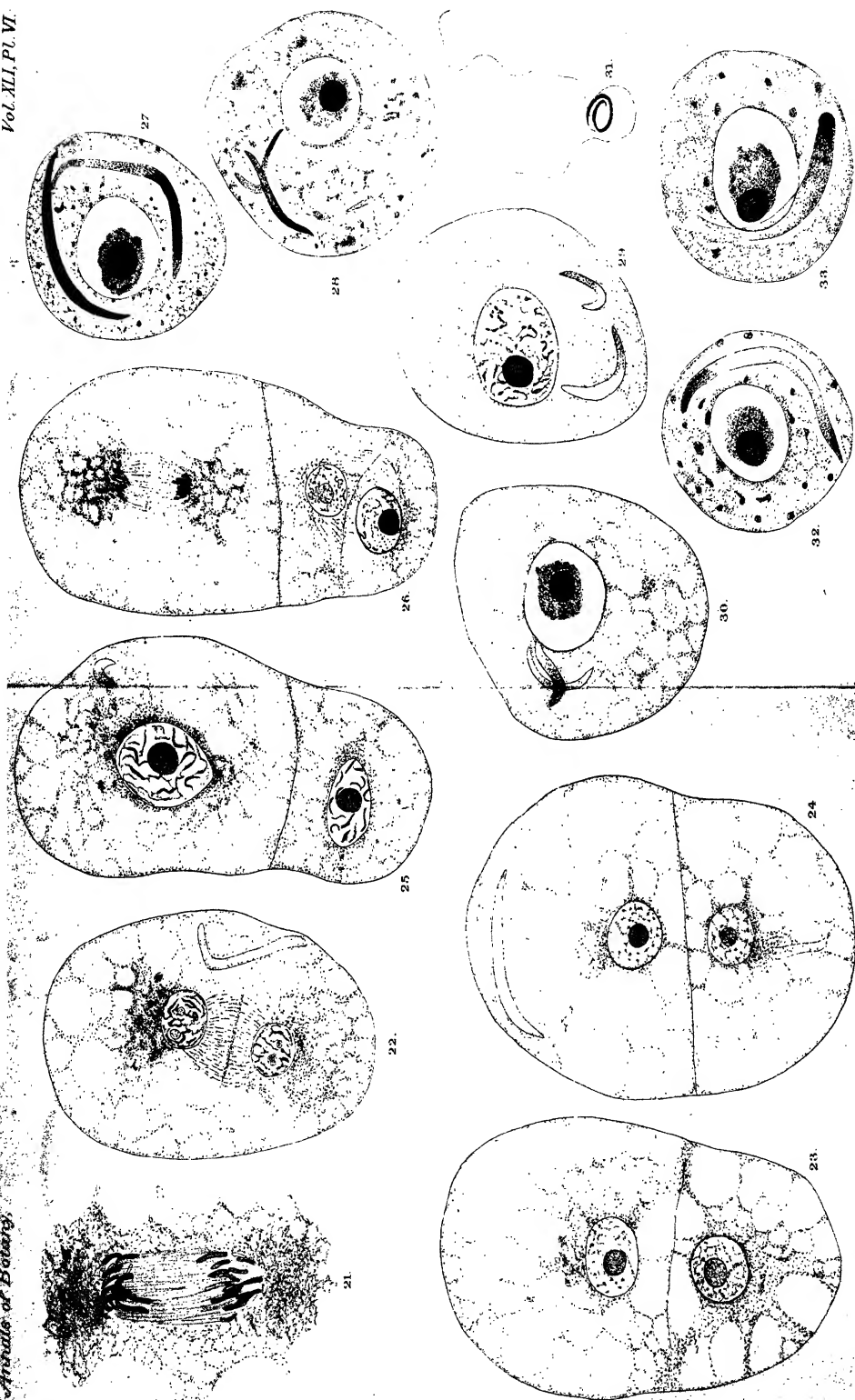
PLATE V.

- Fig. 1. Zygote 6 min. after insemination. $\times 2,100$.
 Fig. 2. Zygote $29\frac{1}{2}$ hrs. after insemination. $\times 2,100$.
 Fig. 3. Zygote showing male nucleus within the female, 42 hrs. $\times 2,100$.
 Fig. 4. Nucleus of same more highly enlarged. $\times 3,200$.
 Figs. 5-7. Fusion nuclei, paternal chromatin at the left, maternal at the right, 42 hrs. $\times 3,200$.
 Fig. 8. Nucleus containing only maternal chromatin of zygote on same thallus as that shown in Fig. 6, 42 hrs. $\times 3,200$.
 Fig. 9. Zygote after nuclear union, paternal chromatin at centre, lower focus, $45\frac{3}{4}$ hrs. $\times 2,100$.
 Fig. 10. Nucleus of zygote on same thallus as Fig. 9, paternal chromatin centre, lower focus, $45\frac{3}{4}$ hrs. $\times 3,200$.
 Fig. 11. Zygote with nucleus showing maternal chromatin at left and above, paternal at right and below, $59\frac{3}{4}$ hrs. $\times 2,100$.
 Fig. 12. Zygote, maternal and paternal chromatin indistinguishable, 84 hrs. $\times 2,100$.
 Fig. 13. Nucleus of another zygote similar to Fig. 12, 84 hrs. $\times 2,400$.
 Fig. 14. Zygote at very early prophase of first division, 168 hrs. $\times 1,700$.
 Fig. 15. Nucleus of same. $\times 3,200$.
 Fig. 16. Nucleus of similar zygote, slightly later prophase, 144 hrs. $\times 3,200$.
 Fig. 17. Nucleus of similar zygote, later prophase, 168 hrs. $\times 3,200$.
 Fig. 18. Zygote at late prophase of first division, 168 hrs. $\times 1,700$.
 Fig. 19. Nucleus of same. $\times 3,200$.
 Fig. 20. Zygote at metaphase, first division, 144 hrs. $\times 1,700$.

PLATE VI.

- Fig. 21. Late anaphase, first division, 168 hrs. $\times 3,200$.
 Fig. 22. Telophase, first division, 192 hrs. $\times 2,100$.
 Fig. 23. Late cytokinesis, first division, 168 hrs. $\times 1,700$.
 Fig. 24. Two-celled embryo, 168 hrs. $\times 1,700$.
 Fig. 25. Prophase, second division, 244 hrs. $\times 1,700$.
 Fig. 26. Telophase, second division, 280 hrs. $\times 1,700$.
 Fig. 27. Egg of *F. angulosa* containing male nuclei of *Riccardia pinguis*, 1 hr. 10 min. $\times 2,100$.
 Fig. 28. Egg of *F. angulosa* containing male nuclei of *Sphaerocarpus Donnellii*, 5 min. $\times 2,100$.
 Figs. 29, 30. Eggs of *F. angulosa* fertilized by antherozoids of *S. Donnellii*, 120 hrs. $\times 2,100$.
 Fig. 31. Antherozoid of *Funaria hygrometrica*. $\times 2,100$.
 Fig. 32. Egg of *F. angulosa* containing male nucleus of *Funaria hygrometrica*, 6 min. $\times 2,100$.
 Fig. 33. Egg of *F. angulosa* containing male nucleus of *Funaria hygrometrica*, 12 days. $\times 2,100$.





I. INTRODUCTION.

IN May, 1924, through the kindness of the Superintendent of the Royal Botanic Garden, Sibpur, Calcutta, I received spirit material of the spikelets of three Bamboos which had flowered under cultivation in the Garden. One of these was identified by the late Mr. J. S. Gamble, F.R.S., to whom I submitted specimens, as *Cephalostachyum virgatum*, Kurz (*Melocanna virgata*, Munro (6)). This species is a rare Burmese Bamboo, which, at the date of Gamble's monograph (3, 1896), had never been collected in British India except by W. Griffith and J. W. Oliver. The flowers are described as possessing three lodicules and six stamens (2), but on looking at microtome series through the Calcutta spikelets, I was astonished to meet with striking variations from this scheme. In my preliminary examination, I cut sections of three spikelets; I found that two of the four flowers included in the sections contained structures resembling lodicules but bearing pollen-sacs, which aroused my interest, because of our uncertainty as to the status of the lodicule. The appearance of these transition organs in two of three spikelets picked out at random suggested that they might probably be frequent in these Calcutta specimens, which perhaps came from a stock that had varied under cultivation. I therefore thought it worth while to cut serial sections of a large number of spikelets, so that I have now been able to follow the structure of seventy individual flowers; these flowers not only show a large number of 'stamen-lodicules', but also various other deviations from the normal. I propose to describe my observations upon them at some length, because abnormalities demand more detailed illustration and comparative description than normal forms, since there is more individual variation among aberrant than typical organs; and it often happens, also, that accounts of abnormalities cannot, for lack of material, be checked and supplemented by other workers. Moreover, although a cursory examination of abnormal structures is liable to give the impression of sheer chaotic anomaly, it is surprising to find how much regularity within irregularity may be revealed by an intensive study.

As I was anxious to examine the wild type-form of the species for comparison with the abnormal Calcutta material, the late Mr. Gamble was so kind as to give me some spikelets from the specimens on which he had based the addenda to his description (3, p. 132); these had been collected by J. W. Oliver at Bhamo, Upper Burma, in March, 1895.

In order to get an idea of the structure of other members of the genus, I have cut sections of *Cephalostachyum Fuchsianum*, Gamble, and of *C. capitatum*, Munro. For spikelets of these two species from British Bhutan (Cambridge Botany School Herbarium) I am indebted to Professor A. C. Seward. This paper represents part of the work carried out with the aid of a grant from the Dixon Fund of the University of London.

2. THE NORMAL STRUCTURE OF THE SPIKELETS OF *CEPHALOSTACHYUM*.

In his description of the spikelets of this genus, Kurz (5) writes, 'some of the lower outer paleas gemmiparous, the buds developing at length into perfect spikelets and supplanting the deflorate or fruit-bearing older ones'. My sections of *C. virgatum* do not as a rule include the structures below the lower fertile flower, but I have seen a bud, such as Kurz describes, in three spikelets. Fig. 1, C₁, p. 50, shows a bud in the axil of an outer empty glume. Its prophyll, *pr.*, has its median bundle in the lateral position facing the midrib of the succeeding leaf, *l*.

The spikelet of *Cephalostachyum* is described as one-flowered, and the species *C. virgatum* as having, in addition, 'one to two small sterile flowers' below the one perfect flower (3). But my observations show that this arrangement is not invariable, and that two fertile flowers may occur: in fact I suspect that this may be common. I cut up my small supply of herbarium material of the wild plant, so as to encourage penetration with paraffin, with the result that the flowers were nearly all isolated. But in one spikelet the lower flower was found to contain a caryopsis, while the upper flower showed a gynoeceium. In the specimens from the Calcutta Garden—which cannot however be used for systematic description because of their abnormalities—two perfect flowers, in each of which the gynoeceium enclosed an ovule, were found in the case of three spikelets.

The individual flowers are borne on the slender rachilla in the axils of flowering glumes (bracts), and each is enclosed in a palea (bracteole) which has two main bundles in a lateral position, and one to three smaller bundles in the median region between the main laterals (*one*, Fig. 1, C₂, D, E₂–E₅, p. 4; *two*, Fig. 2, B, p. 52, and Fig. 4, B₅, p. 61; *three*, Fig. 5, E₃, p. 63). Fig. 1, C₂–C₄ show how, towards the apex, the median bundle of the palea dies out, and the two main laterals supply the two mucros.

The construction which appears to be normal for the flower itself is shown in Fig. 1, D, which is drawn from a wild specimen, while E₁–E₅ are also typical except for the absence of the back stamen. There are three vascular lodicules, one being median and posterior, and the other two lateral and anterior; the bundles are more clearly seen in Fig. 1, F. Between the margins of the lodicules appear the three stamens of the outer whorl (*st.*), while opposite the middle of each lodicule comes a stamen of the inner whorl (*st.*). The gynoeceium has a single ovule attached to the back wall in the median plane; Fig. 1, G, shows an ovule in more detail with its integuments.

For comparison I have included sections of *Cephalostachyum Fuchsianum*, Gamble (Fig. 1, A), and of *C. capitatum*, Munro (Fig. 1, B); in both of these species the flower is constructed on the same lines as in *C. virgatum*.

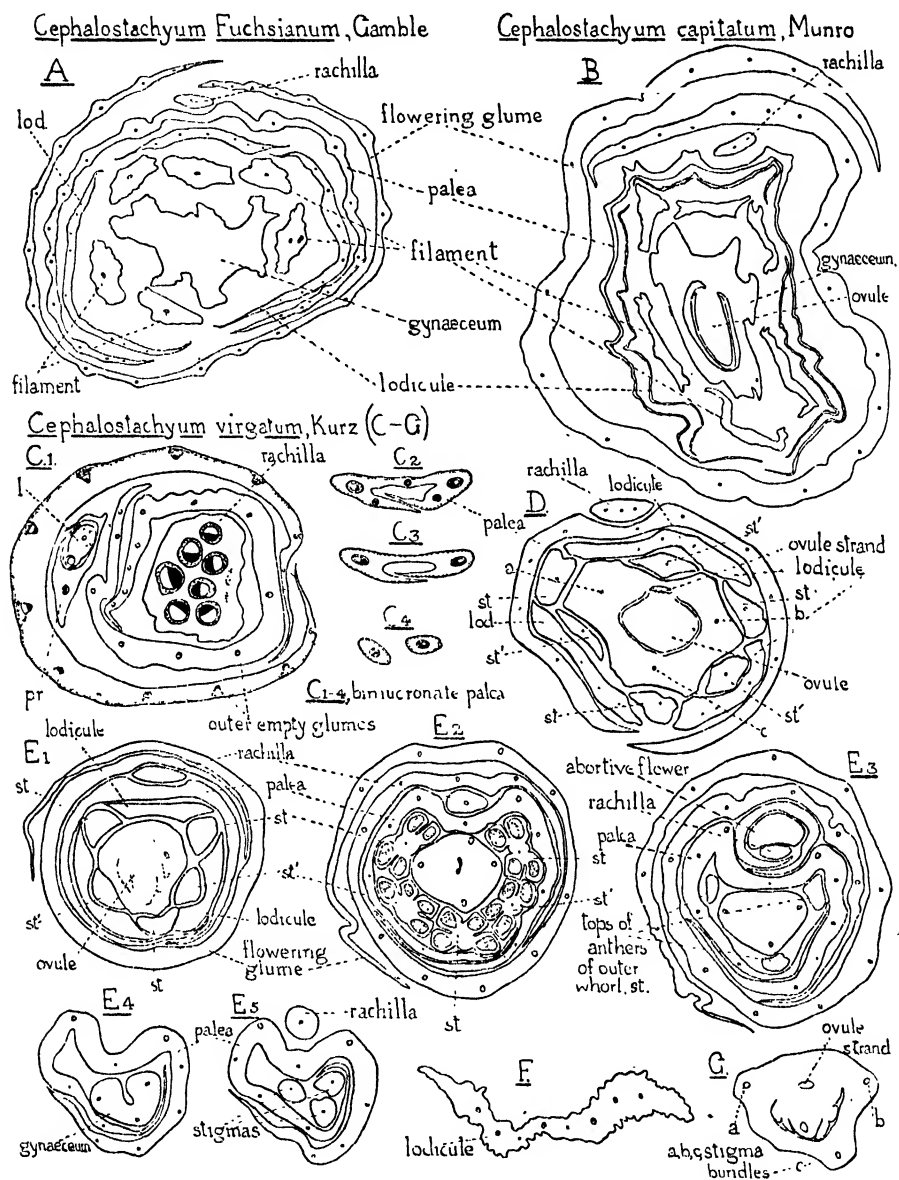


FIG. 1. A, *Cephalostachyum Fuchsianum*, Gamble. Transverse section of flower below level of ovule ($\times 23$), from British Bhutan, Cambridge Botany School Herbarium. B, *C. capitatum*, Munro. Transverse section of flower ($\times 47$), from British Bhutan, Camb. Bot. Sch. Herb. C-G, *C. virgatum*, Kurz. C₁-C₄, transverse sections from a series through a spikelet from Royal Botanic Garden, Calcutta ($\times 47$). C₁, spikelet below flowers, showing two outer empty glumes (preceding the first flowering glume), the lower of which has a bud in its axil with prophyll, *pr.*, and first leaf after prophyll, *l.* (outer glume somewhat broken and reconstructed). C₂-C₄, top of palea from the flower borne by the rachilla in C₁, to show venation and bimucronate tip; sclerized epidermis and bundles with strong fibrous sheaths. D, transverse section of a flower of the wild plant, collected by J. W. Oliver, Bhamo, Burma, March, 1895 ($\times 47$). Drawing made from several sections and somewhat reconstructed, owing to the shrinkage of the herbarium material; *a*, *b*, *c*, strands of outer gynoeceum whorl; the ovule-supply strand is an ill-defined mass of vascular tissue; *st.*, *st.*, *st.*, stamens of outer

The vascular system of the gynoeceium in *C. virgatum* consists of a median posterior ovule-supply strand, and three other stigma-supply bundles—*a* and *b*, which are lateral and posterior, and *c*, which is median and anterior. The ovule strand dies out before the stigmas are reached; the passage of the other three bundles into the stigmas can be traced in Fig. 1, E_3 – E_5 .

The rachilla is continued above the upper fertile flower and bears a flowering glume subtending a rudimentary flower (Fig. 1, E_3 , and Fig. 5, E_3 , p. 63). The rachilla persists for a short distance above this flower rudiment, and in one spikelet I have observed it to give rise to yet another rudimentary flowering glume—in this case enclosing no flower—before dying out.

3. DIVERGENCES FROM THE NORMAL IN THE FLOWERS OF *Cephalostachyum virgatum*, KURZ.

(i) Variation in the Lodicules.

Certain variations from the normal form of *C. virgatum* occur not only under cultivation, but in flowers collected in their native habitat. In sending me spikelets collected at Bhamo, Burma, Mr. Gamble wrote (June 26, 1926), 'Both in number of stamens and of lodicules the species is very variable. I examined one spikelet this morning. It had four stamens instead of six and two lodicules instead of three.' My sections of this material fully bear out Mr. Gamble's statement. I found three lodicules in three flowers, and two in four flowers; one of the latter is drawn in Fig. 2, E, p. 52. And in one of the flowers with three lodicules these structures were unequal in size, one being small and non-vascular (Fig. 2, D). In the Calcutta material, although three lodicules are most frequently present, a considerable proportion of the flowers have only two; this may sometimes be correlated with the presence of the stamen-lodicules which we shall discuss in the next Section, but occasionally it seems to be brought about by simple fusion of the two anterior lodicules (Fig. 2, H, p. 52). I have also met with two flowers in which a fourth lodicule occurred (Fig. 2, B, p. 52, and Fig. 6, B_1 , p. 65). In Fig. 4, C_1 and C_2 , p. 61, there are three lodicules, but two are anterior, the outer one being very wide and enclosing the other, which is much narrower.

In three flowers I have noticed one of the lateral lodicules with its

whorl; st' , st'' , st''' , stamens of inner whorl. E–G, from Calcutta Botanic Garden. E_1 – E_5 , series of transverse sections through the second flower of a spikelet ($\times 47$); E_1 , near base of lodicules and filaments; one lateral lodicule and the front stamen, st' , are united at their extreme base; the ovule is drawn with a dotted line, as it is only visible at a level between E_1 and E_2 . E_2 , cut above the lodicules and through the anthers, at a level at which the ovary cavity has narrowed to a chink; E_3 , at level above the top of the anthers of the inner whorl; E_4 , lobing of gynoeceium preparatory to stigma formation; E_5 , stigmas. F, transverse section of a single front lateral lodicule from another flower ($\times 77$) to show vascular strands. G, transverse section ($\times 77$) of ovary from upper flower of a spikelet to show embryo-sac and rudimentary integuments.

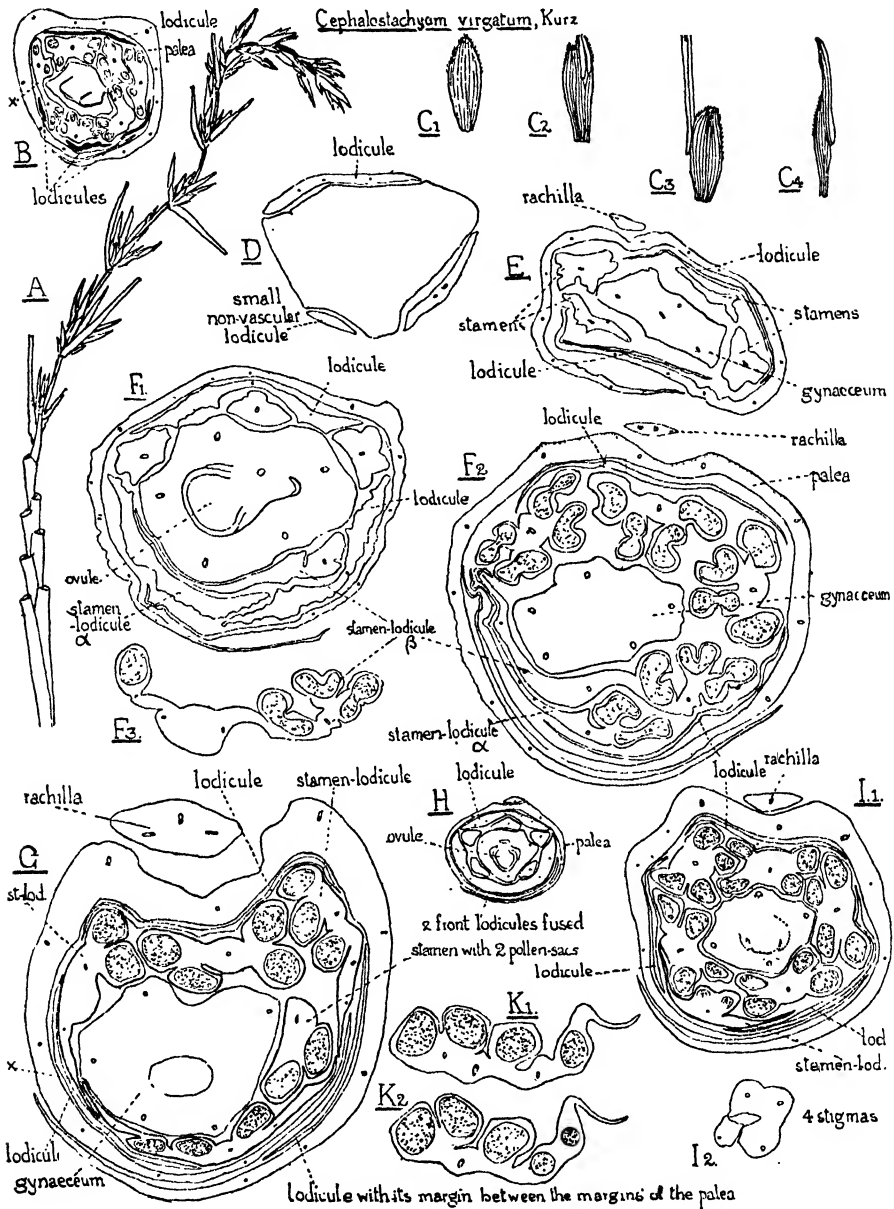


FIG. 2. *Cephalostachyum virgatum*, Kurz. A, part of a flowering shoot from Royal Botanic Garden, Calcutta ($\times \frac{1}{2}$). The partial inflorescences arise in the axils of leaves whose limbs seem to have fallen. B, transverse section of a flower, Cal. Bot. Gard. ($\times 47$), to show four lodicules (black) and a stamen (\times) with a 2-bundled connective; at a lower level the ovary, which contains no ovule, is closed. C₁-C₄, lodicule and stamen-lodicules from dissected flowers, Cal. Bot. Gard.; C₁, normal lodicule, height 3.2 mm.; C₂-C₄, stamen-lodicules; C₂, total height 4 mm., length of pollen-sacs 1.5 mm.; C₃, lodicular part 3.5 mm., pollen-sacs 5.5 mm.; C₄, total height 6.4 mm., pollen-sacs 4.3 mm. D and E, from Bhamo, Burma, J. W. Oliver, March, 1895. D, transverse section of a flower below detachment of stamens, to show inequality of lodicules ($\times 47$). E, transverse section of a flower with two lodicules and four stamens ($\times 47$). F-I, Cal. Bot. Gard. F₁-F₈, transverse

margin between the margins of the palea. In one of these three flowers, which is illustrated in Fig. 3, F_1 - F_3 , p. 55, I was able to trace the relation of the palea and lodicule to the extreme base. It will be seen that the insertion of one margin of the palea inside the margin of a lodicule occurs very early—at a level, indeed, at which the lodicules are only just beginning to be detached. I have previously recorded an example of a similar anomaly in another Bamboo, *Schizostachyum brachycladum*, Kurz (1, p. 460).

(ii) Lodicular Variation in the Androecium.

(a) Free Stamen-lodicules.

The abnormalities described in the preceding paragraphs relate merely to variation in the size, number, and position of the lodicules. A much more interesting divergence from the normal is the occurrence, in the material from the Calcutta Garden, of organs partaking of the characters both of stamens and of lodicules. Fig. 2, C_1 , shows a normal lodicule from a dissected flower, while C_2 - C_4 represent 'stamen-lodicules', in which pollen-sacs forming a more or less complete anther are borne on an organ exactly like a lodicule.¹ These sketches do not in the least exhaust the wide range of forms which may be assumed by the stamen-lodicules. Various types are shown in section in Figs. 2, 3, &c. The commonest form, of which the stamen-lodicules marked α in Fig. 2, F_2 , and Fig. 3, C_1 and C_4 , p. 55, are examples, has a connective which bears two pollen-sacs on one side and a lodicular flap, and sometimes a third pollen-sac, on the other. Three stamen-lodicules are seen in the flower drawn in Fig. 2, G , two of which are of the α type, while the third (anterior) shows three pollen-sacs in a more or less median position with a flap on either side. Variations on the latter type, which is rather rare, can also be seen in Fig. 2, I_1 (anterior *st.-lod.*), and Fig. 5, C_1 , p. 63 (right-hand *st.-lod.*). An unusual form is that marked β in Fig. 2, F_1 - F_3 ; it has three pollen-sacs at one margin and one at the other. It is exceptional to find more than four pollen-sacs in a stamen-lodicule, but Fig. 2, K_1 and K_2 , p. 52, show one which has five, and in another flower I have found an example with six

sections from below upwards through a flower showing stamen-lodicules. G , transverse section of the flower with stamen-lodicules whose base is shown in Fig. 3, F , p. 55 ($\times 77$). H , transverse section of a flower with front stamen absent and two front lodicules fused ($\times 23$). I_1 and I_2 , transverse sections of a flower ($\times 77$); I_1 , whole flower to show stamen-lodicule; ovule drawn with dotted line as it is only visible at a slightly lower level than I_1 ; I_2 , section at a higher level, through gynoecium only, to show four stigmas. K_1 and K_2 , transverse sections lower (K_1) and higher (K_2) in a stamen-lodicule with five pollen-sacs ($\times 47$). This stamen-lodicule seems to arise through fusion of the front stamen with one of the front lodicules; lower down it has a wider lodicular flap.

¹ These sketches are enlarged: the actual dimensions will be found stated in the legend.

54 *Arber.—Abnormalities in Cephalostachyum virgatum, Kurz.*

sacs. Fig. 3, C₁-C₄ (β), E₁, and E₂, p. 55, and Fig. 5, C₂, p. 63, illustrate stamen-locicules which diverge comparatively little from normal stamens.

We have so far considered the stamen-locicules merely as individual organs. For the question of their interpretation we need, however, to go beyond this, and to get an idea of their mode of incidence in the material studied. In the following tables an attempt is made to analyse the characters of the flowers from the Calcutta Garden of which serial sections were cut.¹

The flowers *without stamen-locicules* total thirty-four, and may be classified as follows :

Number of Parts in the Lodicule-androecium System.			Number of Flowers.	References to Figures.
Lodicules.	Stamens.	Stamen-locicules.		
4	6	0	1	Fig. 7, B ₁ -B ₃ , p. 66 ; Fig. 8, A, p. 70.
3	6	0	19 ²	
3	5	0	12	
3	4	0	1	
2	5	0	1	Fig. 2, H, p. 52.

The flowers *with stamen-locicules* total twenty-four, and may be classified as follows :

Number of Parts in the Lodicule-androecium System.			Number of Flowers.	References to Figures.
Lodicules.	Stamens.	Stamen-locicules.		
3	6	1	1 ³	Fig. 2, I, p. 52 ; Fig. 3, D ₁ , D ₂ , E ₁ , E ₂ , p. 55. Fig. 2, K ₁ , K ₂ , p. 52.
3	5	1	5	
2	5	1	5	
3	4	2	1	
3	4	1	2	Fig. 9, A ₁ , p. 71.
3	3	2	1	Fig. 2, G, p. 52. Fig. 7, D, p. 66.
3	3	3	1 ⁴	
3	3	1	1	
3	2	2	1	
3	1	1	1	Fig. 4, A ₁ and A ₂ , p. 61.
2	4	2	2	Fig. 3, B, C ₁ -C ₄ , p. 55.
2	4	1	2	Fig. 3, A ₁ , A ₂ , p. 55.
2	3	2	1	Fig. 2, F ₁ -F ₃ , p. 52.

We see from these figures that whereas twenty out of the thirty-four flowers without stamen-locicules possess the full complement of six stamens, on the other hand six stamens occur in only one of the twenty-four flowers with stamen-locicules. From this we may conclude that there is a correlation between the occurrence of stamen-locicules, and a reduction

¹ I am for the moment altogether omitting twelve flowers in which pollen-sacs were associated with the gynoecium ; these are reserved for separate treatment (p. 59).

² In three of these flowers the sixth stamen was reduced to an abortive filament (Fig. 7, E, p. 66).

³ This flower also contained a small abortive nondescript organ which might have been a stamen or a locicule.

⁴ The third stamen is not shown in Fig. 2, G ; it is an abortive filament (front lateral inner) and at a lower level occupies the position marked x.

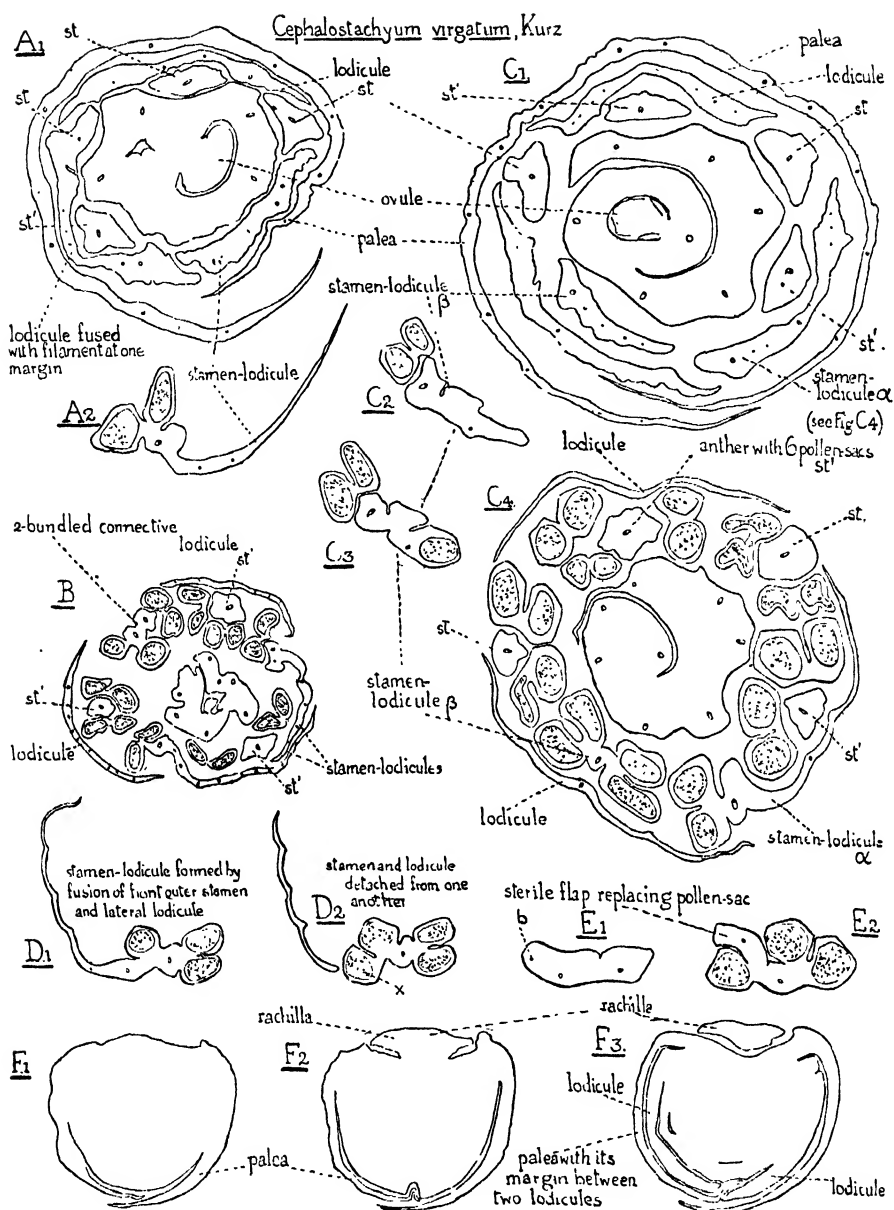


FIG. 3. *Cephalostachyum virgatum*, Kurz, from Royal Botanic Garden, Calcutta. A₁ and A₂ ($\times 47$); A₁, transverse section of a flower with one stamen-lodicule, cut at level of ovule; A₂, stamen-lodicule at a higher level. B, transverse section ($\times 23$) of a flower with two lodicules, two stamen-lodicules, and a stamen with a 2-bundled connective; for a more detailed study of the latter see Fig. 7, H₁–H₃, p. 66. C₁–C₄, transverse sections of a flower with two lodicules and two stamen-lodicules at successive levels from below upwards ($\times 47$); C₁, whole flower and palea; C₂ and C₃, stamen-lodicule β only; C₄, flower (palea not drawn). D₁ and D₂, transverse sections at two levels of a front outer stamen-lodicule to show lodicular flap replaced above by a pollen-sac marked \times ($\times 47$). E₁ and E₂, transverse sections at two levels ($\times 77$) of a front lateral inner stamen with wide filament (E₁) and pollen-sac replaced by a short lodicular flap (E₂). F₁–F₃, transverse sections ($\times 47$) of the extreme base of the flower whose higher region is drawn in Fig. 2, G, p. 52, to show insertion of one palea-margin between two lodicules.

in the number of normal stamens. The idea cannot but suggest itself that stamen-lodicules may, in some if not in all cases, represent missing stamens. To test this notion, it is necessary to turn to a subject upon which we have not yet touched—namely, the positions occupied by the stamen-lodicules within the individual flowers. The result of a scrutiny of the material from this standpoint is to show that the stamen-lodicules do, in fact, nearly always occupy the position of absent stamens. My observations on the twenty-four flowers possessing stamen-lodicules may be tabulated as follows :

<i>Number of Stamen- lodicles.</i>	<i>Position of Stamens whose places are taken by the Stamen-lodicules.</i>	<i>Number of Flowers.</i>	<i>References to Figures.</i>
1	Front outer.	11	Fig. 2, I ₁ , p. 52 ; Fig. 3, A ₁ , p. 55 ; Fig. 9, A ₁ , p. 71.
1	One back lateral outer.	3	Fig. 4, A ₂ , p. 61 ; Fig. 7, D, p. 66.
2	Two back lateral outer.	1	
2	One back lateral outer and front outer.	1	Fig. 3, B, p. 55.
3	Two back lateral outer and front outer.	1	Fig. 2, G, p. 52.
2	One back lateral outer and one front lateral inner.	2	
2	Front outer and one front lateral inner.	2	Fig. 2, F ₁ -F ₃ , p. 52 ; Fig. 3, C ₁ -C ₄ , p. 55.
1	One front lateral inner.	1	Fig. 3, E ₁ , E ₂ , p. 55.
1	One back inner, jointly with a non- lodicular stamen with two pollen- sacs.	1	
1	No stamen replaced, as the stamen- lodicule is additional to six stamens.	1	

It will be apparent from this table that in the twenty-four flowers to which the numbers relate, twenty-one show one or more stamens of the outer whorl replaced by stamen-lodicules.¹ The stamen which most frequently suffers this change is the front median member of the outer whorl, which is lodicular in fifteen flowers. In four flowers one of the lateral inner stamens is transformed as well as one of the outer whorl, and in one flower it is a lateral front inner stamen alone that becomes partially sterile (Fig. 3, E₁ and E₂, p. 55). My only example of a lodicular stamen which is undoubtedly the back member of the inner whorl is from a flower not tabulated above, since it belongs to the group which will come under consideration on p. 59 (Fig. 5, C₁, C₂, p. 63).

Referring again to the table on p. 54, we see that only one of the thirty-four flowers without stamen-lodicules shows a reduction in the number

¹ Four of the twelve flowers showing stamen-lodicules associated with the gynoecium, which we shall consider on p. 59, have in addition free stamen-lodicules. The stamens replaced by these free stamen-lodicules are, as far as can be judged: two outer stamens in two flowers; one outer stamen in one flower; one outer and one inner stamen in one flower.

of normal lodicules, and this reduction is apparent rather than real, since there has merely been a fusion of the two front lodicules into one (Fig. 2, H, p. 52). But, on the other hand, ten of the twenty-four flowers with stamen-lodicules have only two normal lodicules, while fourteen have the usual three. So there seems to be some correlation here between the occurrence of a stamen-lodicule and the loss of a normal lodicule. We are thus confronted with the question of what part, if any, the normal lodicules play in the production of stamen-lodicules. We may dismiss at once the fourteen flowers which possess the full equipment of three lodicules and also one or more stamen-lodicules, for in none of these can the stamen-lodicules be held to replace normal lodicules (e.g. Fig. 2, G and I₁, p. 52). But the structural plan of the ten flowers which have stamen-lodicules, but only two normal lodicules, demands further study. On looking through them from this point of view, I have found that in all of them the flap of a stamen-lodicule occupies the position which the absent normal lodicule would ordinarily take. In such a flower, for example, as Fig. 3, C₄, p. 55, where there are five stamens, and the half anther of stamen-lodicule α fills the place of the missing sixth stamen, its lodicular flap occupies the position of the missing third lodicule. It seems to me most probable that in such cases we are dealing with a more or less reduced stamen and an adjacent lodicule which have fused completely. The occurrence of such a fusion as a local feature is shown in Fig. 3, A₁, p. 55, in which a front lodicule is fused basally with the adjacent filament, *st.*, from which it becomes free at a higher level. Fig. 3, D₁ and D₂ represent a stamen-lodicule in which the compound character is seen particularly plainly, since the stamen-member has retained its four pollen-sacs, and the lodicule-member, though united with it below, becomes detached at a higher level. One cannot, however, be certain of the compound or simple character of a stamen-lodicule merely from its form, for in another flower I have found a stamen-lodicule of purely staminal origin whose structure exactly recalls Fig. 3, D.

In one of the flowers which does not belong to those tabulated above, but is discussed in Section (c), p. 59, the two front lodicules and the front stamen have apparently fused into a single member (Fig. 6, C₁-C₃, p. 65).

To sum up the results of the present Section of this paper, I may say that I have examined altogether 38 free stamen-lodicules belonging to 27 flowers, and I find that they fall into three classes: 27 of them each represent 1 *stamen*; 10 of them each represent 1 *lodicule* + 1 *stamen*; and 1 of them represents 2 *lodicules* + 1 *stamen*. More than one type may occur in the same flower. For example, in Fig. 2, F₂, p. 52, stamen-lodicule α may be held to represent the missing third lodicule fused with the front outer stamen, while stamen-lodicule β represents a front inner stamen, pure and simple. It is not possible to say with certainty to which types the stamen-lodicules, whose external appearance is shown in Fig. 2, C₂-C₄, p. 52,

should be referred, since it is a point which cannot be settled without the aid of serial sections of the whole flower.

(b) *The Significance of the Free Stamen-lodicules in Relation to the Morphology of the Grass Flower.*

There has been endless discussion about the nature of the lodicules in the flower of the Gramineae, much of which might probably have been spared if botanists' basic ideas about this family had been derived from the Bamboos instead of from the reduced Grasses of temperate countries. I do not think that it is worth while to give an historical account of this voluminous controversy, since the greater part of it is of antiquarian rather than of scientific interest. The view taken by Hackel in the 'Pflanzenfamilien' (4), which seems to have had considerable influence with British botanists, is that the Grass flower is naked; the palca is regarded as the first bracteole; the two front lodicules together as the second bracteole; and the back lodicule (where it exists) as a third bracteole. On the other hand Schuster (8) and others have maintained that the lodicules represent an inner perianth whorl.

We have now to consider whether the abnormal flowers here described afford any evidence towards the solution of the problem of whether the lodicules are of bract- or of perianth-nature. I have shown that 'stamen-lodicules'—organs resembling lodicules, but bearing pollen-sacs—occurred in flowers of *Cephalostachyum virgatum* under cultivation at Calcutta, and that these stamen-lodicules might result from the fusion of either one or two lodicules with a stamen, or might be purely staminal—the stamen in the latter case becoming partially sterile and the sterile regions closely resembling a lodicule. The only reference I have found in the literature to anything of the same kind is J. S. Gamble's statement (3) that another Bamboo, *Schizostachyum latifolium*, Gamble, has three or four lodicules, 'the fourth apparently a modified stamen'; I hope that I may have an opportunity later of examining this species.

The existence of lodicular stamens may not in itself *prove* the perianth-nature of the lodicules, but I think that it lends probability to this view. For there is undoubtedly a much closer affinity between stamens and perianth members than between stamens and bracts, and thus the existence of members transitional between stamens and lodicules may fairly be treated as an indication that the lodicules are more likely to be of perianth-nature than of bract-nature.

(c) *Stamen-locicules associated with the Gynoecium.*

In the discussion of stamen-locicules in the preceding Sections of this paper, I have left out of account twelve flowers, belonging to the same consignment of material from the Calcutta Botanic Garden, in which from one to four pollen-sacs occur in association with the gynoecium. I have drawn this distinction—although I myself regard them as probably falling into the same category as the flowers with free stamen-locicules already considered—because their interpretation is a matter in which there is room for difference of opinion. For the same reason, instead of calling the structures in question ‘stamen-locicules’ in Figs. 4, 5, and 6, I have labelled them with the non-committal name, ‘flanges bearing pollen-sacs’.

Fig. 4, B₂, p. 61, and Fig. 5, C₁ and D, p. 63, may serve to indicate the rather surprising appearance often presented by the flowers which we are considering, when seen in section. In each case one or more pollen-sacs are embedded in a flange of tissue which is continuous with the gynoecium wall. In Fig. 5, D, the ‘gynoecium pollen-sac’ appears to be abortive, but in Fig. 4, B₂, it shows tapetum and pollen mother-cells (B₂), and is at precisely the same stage of development as the normal stamens in the same flower. If these were the only flowers available showing pollen-sacs associated with the gynoecium, it would be difficult to avoid the conclusion that we had to do with staminody of the carpels. But there are other examples which throw a somewhat different light on the matter. Fig. 5, A₁, p. 63, is of special interest in this connexion. Here we see the filaments of five stamens—three outer (*st.*) and two inner (*st'.*)—occupying their normal positions in relation to the three locicules. Attached to the anterior lateral face of the gynoecium is a flange which, in the region marked x—where the front inner stamen ought to be—shows a localized area of tissue including a single bundle, which exactly recalls the five filaments in the appearance and staining quality of its tissue; in the sketch this area is marked off by dotted lines. At the higher level shown in Fig. 5, A₂, the flange has become entirely detached from the gynoecium, and has developed three pollen-sacs at one margin and a fourth at the margin by which it was connected with the gynoecium. That the flange with its pollen-sacs represents the missing sixth stamen, and is thus a stamen-locicule fused in its lower region with the gynoecium wall, seems scarcely open to doubt. It may be compared with stamen-locicule β in Fig. 2, F₁–F₃, p. 52, which also replaces a front inner stamen, and it agrees, even in detailed structure, with Fig. 5, A₂, having three pollen-sacs at one margin and a fourth at the other. An example closely comparable with Fig. 5, A, is drawn in Fig. 6, A₁, p. 65. Here again there are five stamens in addition to a front lateral flange attached to the gynoecium. The identity of part of this flange with a filament is even more obvious here

than in Fig. 5, A_1 , p. 63, because the shape of the filament is partly retained. A little higher up (Fig. 6, A_2), the stamen-locule becomes detached and bears a pollen-sac, while still higher the number of pollen-sacs is increased to four, and there has been a recurrence of fusion with the gynoeceum (Fig. 6, A_3). Still higher the stamen-locule is again detached from the gynoeceum, whose three stigmatic lobes can be recognized in Fig. 6, A_4 . It is the development of the normal number of stigmas in such gynoeccia which furnishes, I think, the best proof that the flange bearing pollen-sacs is not produced at the expense of the gynoeceum itself, but is a foreign member partially fused with it. Fig. 4, B_1-B_5 , Fig. 5, E_1-E_3 , p. 63, and Fig. 6, B_1-B_3 , p. 65, represent other examples, in which, after the detachment of a flange with pollen-sacs, three normal stigmas are formed. In the present connexion we have to rely upon the stigmas because the flowers in this material are frequently sterile, so that no special conclusion can be drawn from an abortive condition of the ovary. It is interesting however to observe, in Fig. 4, C_1 , the occurrence of an ovule in a gynoeceum with a flange which at a higher level (C_2) becomes detached and bears pollen-sacs.

To regard the 'flange bearing pollen-sacs' as a stamen-locule does not present much difficulty in flowers such as Fig. 5, A , and Fig. 6, B , in which the flange becomes detached at an early stage, thus revealing its independence of the gynoeceum. The degree of attachment between the flange and the gynoeceum may however be much closer than this, so that the detachment does not occur until the level of the stigmas, and the individuality of the stamen-locule is hence somewhat obscured. Fig. 6, C_1-C_6 , p. 65, is a case in point. In C_3 the organ consisting of *gynoeceum + flange* is supplied by eight bundles whose history can be followed in C_3-C_5 . In C_4 we still see the flange, which at the base of the stigmas has not yet achieved its freedom. In C_5 the four stigmas are reached, but the flange has died out below this level. If it were not for the comparison with flowers in which the discreteness is more apparent, the compound character of the *gynoeceum + flange* might easily remain unrecognized, and the whole structure might be mistaken for a gynoeceum bearing pollen-sacs.

If we are to regard the flanges bearing pollen-sacs as stamen-locules, however intimate their association with the gynoeceum may be, it becomes important to see how far their position within the individual flowers accords with this view. I find that nine of the twelve flowers in question possess less than six stamens, and that in these flowers the flange seems to replace one of the missing members. It is not always easy to determine the exact position of the flange in the plan of the flower. But, as far as I can judge, in either three or four flowers the flange replaces a front lateral stamen of the inner whorl; in three flowers, the front outer stamen; in two flowers, either the front outer or a front inner. It will be noticed that in two flowers

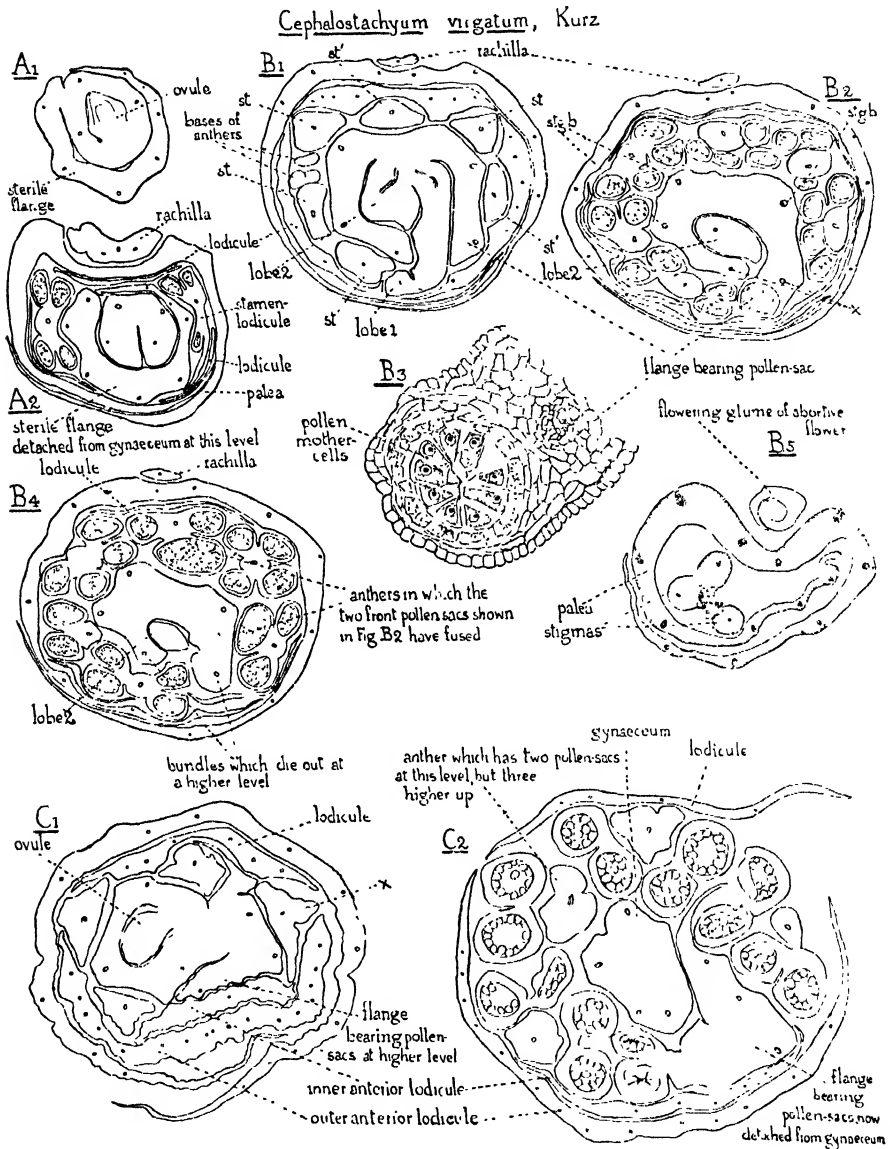


FIG. 4. *Cephalostachyum virgatum*, Kurz, Royal Botanic Garden, Calcutta. A, transverse sections ($\times 47$) of a flower with one stamen-lodicule; A₁, lower, A₂, higher; A₁, gynoecium showing ovule and sterile flange; A₂, whole flower showing sterile flange detached. B₁, B₂, B₃, B₄, B₅, series of transverse sections through a flower ($\times 47$) from level of filaments (B₁) to stigmas (B₅). The projection marked *lobe 1* in B₁ separates from the rest of the gynoecium and forms a free downward prolongation; *lobe 2* is a projection into the interior of the ovary; I find no ovule, but I cannot be sure of its absence, as my series of sections is imperfect. The two bundles marked \times in B₂, die out higher up. B₃, pollen-sac from the flange in B₂ ($\times 198$). C₁ and C₂, transverse sections ($\times 47$), lower (C₁) and higher (C₂) in a flower with two anterior lodicules, and pollen-sacs in a flange from the gynoecium wall; palea omitted in C₂. The stamen marked \times in C₁ is wide at the base of the filament, but normal above.

in which an elongated flange is assumed to replace the front outer stamen (Figs. 5, D, p. 63, and 6, B, p. 65), the lodicular part, which more or less enwraps the gynoeceum, lies *inside* the stamens of the *inner* whorl. This may be held to invalidate my view that in these two cases the flanges are equivalent to *outer* stamens. But I think that the anomaly of their position may, on the other hand, be merely a mechanical result of the connexion between the base of the flange and the gynoeceum, which might prevent the flap of the stamen-lodicule from assuming its rightful place. A comparable example would be the anomalous insertion of a palea margin between two lodicules to which I have referred on p. 53 (Fig. 2, G, p. 52, and Fig. 3, F₃, p. 55).

In the nine flowers discussed there is little doubt that the 'flange bearing pollen-sacs' replaces a stamen—though there may be some doubt as to *which* stamen. But we have still to consider the remaining three flowers in which the flange cannot be treated as replacing one of the regular members of the gynoeceum, since they are all otherwise accounted for. In two of the flowers the flange is additional to the full apparatus of three lodicules and six stamens. In the third flower (Fig. 6, C₁-C₅) I think that it must again be regarded as an extra. Fig. 6, C₁, shows five stamens—the absent one being the front member of the outer whorl—while the two front lodicules are fused edge to edge. In C₂ and C₃, the fused pair of lodicules is found to bear pollen-sacs in the median region, so that it seems reasonable to regard the resulting organ as a front median stamen-lodicule, which has arisen through fusion of the front outer stamen with the two front lodicules. The stamens and lodicules are thus all accounted for, and the flange attached to the gynoeceum and bearing pollen-sacs must be regarded—as in the two preceding flowers—as an extra stamen. It will be recalled that when considering the free stamen-lodicules, we came upon one example showing three lodicules, six stamens, and one stamen-lodicule; here again the stamen-lodicule must be regarded as representing a seventh stamen. To suppose the occasional existence of a seventh stamen, or equivalent organ, in a Bamboo flower is no great assumption when we remember that one of the Bambuseae, the genus *Melocanna*, is defined as having 'stamens five to seven' (3).

That the 'flanges bearing pollen-sacs' associated with the gynoeceum are, in reality—like most of the free stamen-lodicules—equivalent to single stamens, seems to me to be, on the whole, the best interpretation of these structures. But, as I pointed out at the beginning of this Section, I do not feel that I have proved this point conclusively except for one or two individual cases; the possibility that these flanges are really of gynoeceum origin cannot be said to be altogether excluded. There are certain peculiar features in some of the flowers which may perhaps be taken to indicate a liability to flange-development in the gynoeceum itself. In Fig. 4, A₁,

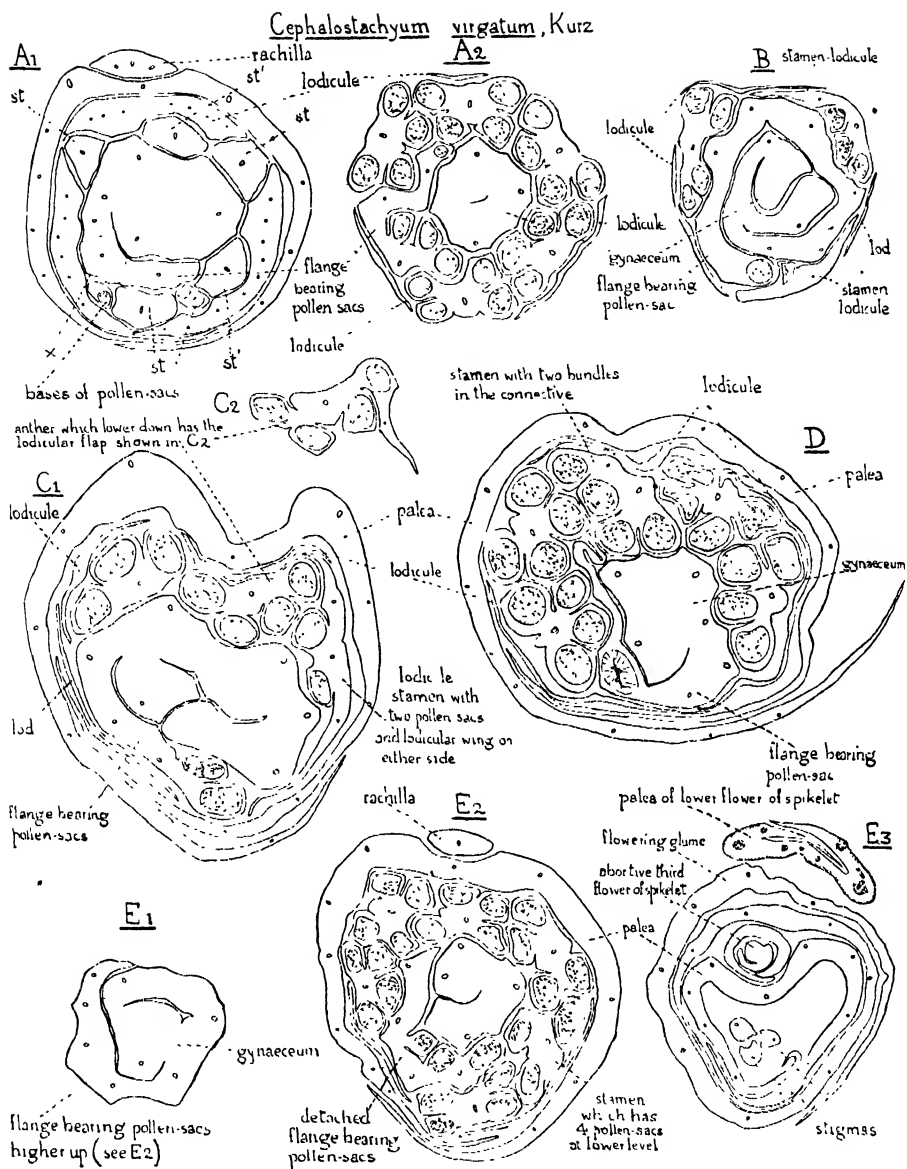


FIG. 5. *Cephalostachyum virgatum*, Kurz, Royal Botanic Garden, Calcutta. A₁ and A₂, transverse sections lower (A₁) and higher (A₂, palea omitted) through a flower which has a flange bearing pollen-sacs attached to the gynoeceum wall ($\times 77$); st., st., st., stamens of outer whorl; st', st', st', stamens of inner whorl; the tissue at the corner marked \times is filament-like in the area enclosed by dotted lines. In A₂ the flange has become free and developed four pollen-sacs. B, transverse section ($\times 77$) of a flower with three lodicules and two stamen-locules; the stamen-locules which have three and one pollen-sacs at this level have respectively four and two higher up; there is a pollen-sac in a flange from the gynoeceum wall. C₁, transverse section of a flower ($\times 77$) to show stamen-locules, and pollen-sacs in flange from gynoeceum; there is an ovule at a level lower than C₁; C₂, back stamen of C₁ at a lower level to show lodicular flap ($\times 77$). D, transverse section ($\times 47$) of a flower with abortive pollen-sac in flange from gynoeceum wall. E₁-E₃, transverse sections through a flower with flange bearing pollen-sacs attached to gynoeceum wall. E₁, gynoeceum only at low level ($\times 77$); E₂, flower at higher level at which flange has become detached ($\times 77$). E₃, the same spikelet at a higher level ($\times 47$) showing stigmas of flower drawn in E₂, and also palea of lower flower, and flowering glume and palea of abortive third flower above.

p. 61, for instance, there is an ovary with a normal ovule, but there is also a sterile flap enwrapping the ovary and attached to the gynoeceum wall. Fig. 4, A₂, shows the gynoeceum at a higher level at which the flap has become free. Still higher, the central bifid lobe, which is the continuation of the ovule-containing part of the gynoeceum, forms a flat two-bundled tip, which seems to be equivalent to two stigmas, while the free flange narrows gradually upwards and also terminates in two stigmas.

(iii) Non-locular Variation in the Androeceum.

We have now considered those variations in the material of *Cephalostachyum virgatum* from the Calcutta Garden in which there are transitions between stamens and lodicules. But the androeceum shows, in addition, a number of other departures from the normal, which on analysis prove to be of some interest ; these will be described in the following paragraphs.

(a) *Bifurcation of Stamens.*

Bifurcation of a stamen seems to be a rare occurrence in these flowers ; the best example I have met with is shown in Fig. 7, B, p. 66. Here one member (a) of the front inner whorl displays a doubleness, which, as the flower contains its complement of six stamens, may reasonably be attributed to chorisism. This stamen has two bundles in the bilobed filament (Fig. 7, B₁) ; higher up it retains the two bundles and develops six pollen-sacs, two of which belong to the smaller lobe of the filament, while still higher the bundle of the smaller lobe dies out (B₂). A seventh pollen-sac originates as a distinct lobe and then divides into two, thus giving eight pollen-sacs ; just above this level the surviving bundle bifurcates, producing two strands with their phloems facing one another (B₃). It is rather curious that the outer one of these two anthers is developed exclusively from the main lobe of the filament, which also supplies both the connective bundles, and two of the four sacs of the inner anther—the minor lobe supplying nothing but the remaining two pollen-sacs of the inner anther. This seems to confirm the idea that we are here dealing with a case of branching rather than of fusion of two stamens.

Another less striking example of bifurcation is shown in Fig. 7, G, p. 66. Here we have an anther with four pollen-sacs, but with a broad two-bundled connective, which divides into two higher up. In one other flower I have met with a stamen closely similar to the one drawn in Fig. 7, G. The stamen with a two-bundled connective shown in Fig. 7, D, also looks as if it belonged to the same type, but I could not follow it to the apex.

There are, on the other hand, occasional two-bundled stamens in which all signs of doubleness are lost before the apex of the anther is reached. It is impossible to say with certainty how such stamens should be classed,

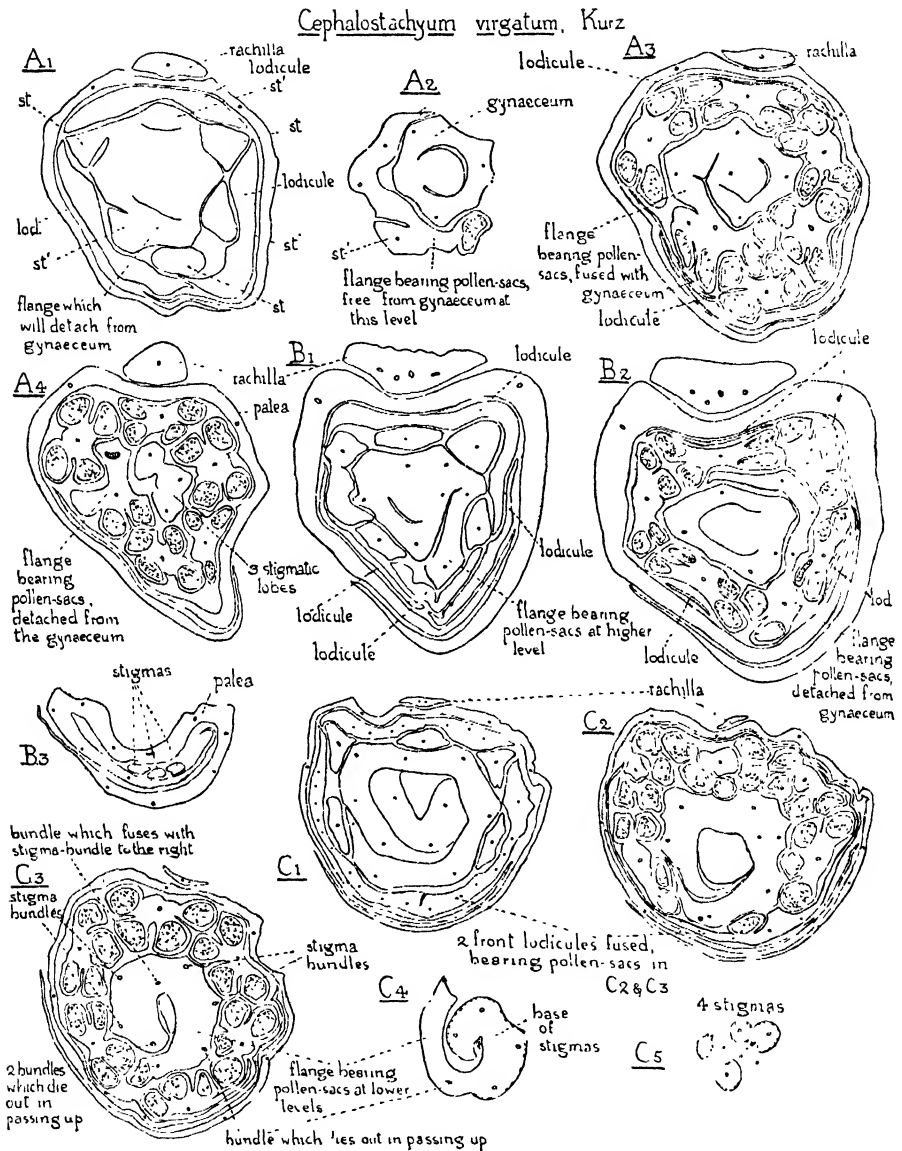


FIG. 6. *Cephalostachyum virgatum*, Kurz. Royal Botanic Garden, Calcutta. A₁–A₄, series of transverse sections ($\times 77$) from below upwards through a flower with flange bearing pollen-sacs attached to the gynoeceum wall; bundles, except main bundles, omitted in palea; A₁, gynoeceum and flange only; there is no ovule. B₁–B₃, transverse sections from below upwards through a flower with gynoeceum flange bearing pollen-sacs; four lodicules; no ovule. B₁ and B₂ ($\times 77$); B₃ ($\times 47$). C₁–C₅, series of transverse sections from below upwards through a flower with flange bearing pollen-sacs fused with gynoeceum up to level at which the stigmas become free. In C₁ the anterior lodicules are fused; in C₂ these united lodicules bear one pollen-sac; in C₃, three pollen-sacs.

though their peculiarities may perhaps indicate a tendency to bifurcation. An example is shown in Fig. 3, B, p. 55, and its history can be followed in Fig. 7, H₁–H₃. Here the filament has two bundles, but one of them,

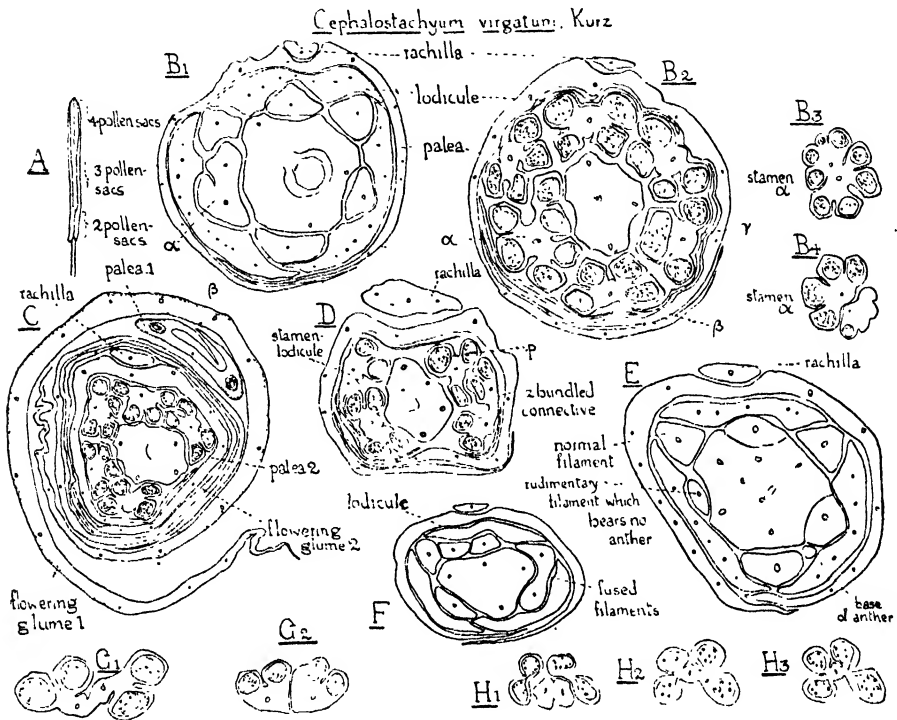


FIG. 7. *Cephalostachyum virgatum*, Kurz, Royal Botanic Garden, Calcutta. A, stamen from a dissected flower showing pollen-sacs varying in number at different levels. The anther was 5.4 mm. long; it had four pollen-sacs at the apex; three in the median region by fusion of two; and two lower down by dying out of the fused pair. B₁–B₄, series of transverse sections through a flower showing stamen-abnormalities ($\times 47$); stamen α has 2-bundled filament in B₁, one bundle and six pollen-sacs in B₂, and in B₃ and B₄ develops into twin anthers; stamen β has two bundles in the filament, but one bundle dies out below the anther; stamen γ has the two front pollen-sacs fused into one for part of their course, but free higher up. C, transverse section of a spikelet ($\times 47$) showing flowering glume 1 and palea 1 of lower flower, and passing through the anthers and above the ovule of the higher flower. (Outer surface of f.g. 1 somewhat damaged and reconstructed.) D, transverse section ($\times 47$) of a flower with one stamen-lodicule and one stamen with a 2-bundled connective. The pollen-sac marked p divides into two in passing up. The two smaller bundles in the gynoeceum die out in passing up. E, transverse section ($\times 77$) at level of filaments of a flower in which one stamen is small and develops no anther. For higher sections of the same flower see Fig. 9, B₁–B₄, p. 71. F, transverse section of a flower at the level of the filaments to show irregularly placed stamens, two of which are united by their filaments ($\times 47$). G₁ and G₂, transverse sections lower (G₁) and higher (G₂) through a back outer stamen with 2-bundled connective whose anther bifurcates ($\times 47$). H₁–H₃, transverse sections ($\times 23$) at three levels in the anther with 2-bundled connective shown in Fig. 3, B, p. 55, which is cut at a level between H₁ and H₂; H₁, base of anther with 2-bundled filament; H₂, anther with one bundle in the connective and one at base of a pollen-sac; H₃, higher in the anther at a level at which the second bundle has disappeared.

though it enters the connective, passes to the base of the pollen-sac and then dies out, so that the anther is normal in its upper region. In Fig. 5, D, p. 63, again, the back stamen, which has two bundles in the connective,

becomes normal and one-bundled at a higher level. The filament marked β in Fig. 7, B₁, has two bundles, the second of which is not visible in the sketch; the extra one dies out below the anther (Fig. 7, B₂).

(b) *Increase in Number of Pollen-sacs.*

A small proportion of the stamens observed had five or six pollen-sacs; the extra sac sometimes arose by division of one of the normal four, and sometimes by development of a distinct lobe. I have found four flowers each with a stamen with six sacs; in three of these the stamen in question was a back stamen of the inner whorl (e. g. Fig. 3, C₄, p. 55), while in one it was a lateral front member of the inner whorl. In the case of three flowers, I have found stamens with five sacs, and in each of these flowers it was the back inner stamen which was so modified. We thus have seven flowers showing stamens with five or six pollen-sacs, the peculiarity being confined to the inner whorl; in six flowers it is shown by the back inner stamen, and in one flower by a front lateral inner stamen.

(c) *Reduction in the Androecium.*

In the material of *Cephalostachyum virgatum* from the Calcutta Garden there is definite evidence for the truth of the usual assumption that the androecium of the Bamboos consists of two three-membered whorls. I find that there is a distinct difference in height between the group made up of the front and two back lateral stamens (theoretically the outer whorl) and the three stamens which alternate with them (theoretically the inner whorl). There were nineteen flowers which had either five or six stamens and no stamen-lobicles, in which I could follow the anthers to their apices. In fifteen of them I found that the three stamens of the outer whorl were taller than those of the inner whorl (cf. Fig. 1, E₂ and E₃, p. 50).

The Calcutta flowers also show a strong tendency to reduction in the number of stamens below the normal six, and this tendency specially affects the inner whorl. In three of the flowers without stamen-lobicles I have found an interesting stage in this reduction—five normal stamens, and a sixth stamen which consists of a rudimentary filament without an anther (Fig. 7, E, p. 66); in each of these three flowers the abortive stamen is one of the lateral front members of the inner whorl. In another flower (Fig. 2, G, p. 52), which had the three outer stamens transformed into stamen-lobicles, one of the front lateral inner stamens again consisted of an abortive filament, which occupied the position marked \times .

The next stage in reduction is represented by the flowers in which we have five stamens only (and no stamen-lobicles). I have met with twelve flowers answering to this description. I find that in two of them it is

impossible to say which stamen is absent; in a third it is the back stamen of the inner whorl (Fig. 1, E₁–E₃, p. 50); and in the remaining nine, it is one of the front lateral stamens of the inner whorl (e. g. Fig. 7, C, p. 66). In the fifteen flowers without stamen-lodicules in which one stamen is either absent or abortive, we thus find that, in no less than twelve, it is one of the front lateral stamens of the inner whorl that is affected, while there are no cases of absence of members of the outer whorl.

In addition to the instances, just detailed, of actual disappearance of a stamen, or of its functional part, I have met with examples of partial reduction, which may represent stages towards elimination. I have found, for instance, that the fusion of two pollen-sacs into one for part of their course is not uncommon. Fig. 7, A, p. 66, shows the external appearance of a stamen modified in this way; it has four pollen-sacs at the apex, three in the median region, and two at the base. The stamen γ in Fig. 7, B₂, and two stamens of the inner whorl in Fig. 4, B₄, p. 61, show similar fused pollen-sacs in section. The most striking example of this peculiarity which I have observed is that of a flower in which the three outer stamens were normal throughout, but the other three behaved in the following manner. In the back inner stamen, two side sacs fused at the extreme base; higher up there were four distinct sacs; then the two front sacs fused; higher still the two back sacs alone survived, the fused sacs having died out, but a third front sac made its appearance close to the apex. One of the front lateral inner stamens had four pollen-sacs, of which the two front members merged into one near the base, and then died out, leaving only the two back ones at the extreme base; this is the type of anther whose external features are indicated in Fig. 7, A. The other front lateral inner stamen had four sacs at the base, but the two front sacs fused in passing up, while at the extreme tip the anther again had four sacs. It is not necessary to give details of other examples: I will only say that I have recorded the fusion of pollen-sacs in one or more stamens in eight flowers, and that in only one of these flowers a member of the outer whorl is affected, while certainly in six, and probably in seven flowers, it is stamens of the inner whorl which show this peculiarity.

The next degree of reduction beyond the fusion of pollen-sacs for part of their length seems to be a *permanent* reduction in the number of pollen-sacs. In five flowers I have found an example of a stamen with three pollen-sacs, but in most of these flowers it was impossible to locate the stamen with certainty. Stamens with two pollen-sacs, however, have fortunately yielded more definite information. I have met with them in ten flowers, though I cannot in all cases be quite certain that the anther has only two sacs throughout its length. In one of the ten flowers it is impossible to say which stamen is affected, as a stamen with two sacs occupies the position of the back stamen, jointly with a stamen-lodicule; in a second

flower the stamen with two sacs is a back member of the outer whorl; while in the remaining eight it is one of the two front lateral inner stamens (e. g. Fig. 2, G, p. 52; Fig. 4, B₄, p. 61).

(d) *The Significance of Non-lodicular Stamen-variation in Relation to the Morphology of the Grass Flower.*

We may conclude from the observations just recorded that there is a strong tendency to diminution in the number of stamens in the material of *Cephalostachyum virgatum* from the Calcutta Garden, and that the stamens of the inner whorl—more particularly the two lateral front members—show a far greater inclination to reduction than the stamens of the outer whorl. The reduction seems to involve the following stages: fusion of pollen-sacs; diminution in number of pollen-sacs; loss of anther; total elimination. But I cannot say whether these observed stages form a real sequence. The liability to degradation and disappearance shown by the inner stamens is probably to be correlated with the crowding due to the lodicules which lie exactly outside this whorl of the androecium. That the lodicules and the stamens of the inner whorl exert considerable mutual pressure is demonstrated by such examples as Fig. 1, E₁, p. 50, in which the two filaments marked *st'* have made grooves for themselves in the upper surfaces of the corresponding lodicules. The back inner stamen seems less liable to reduction and arrest than the other two, but it is possible that the tendency which it occasionally shows towards an increase in the number of pollen-sacs (p. 67) may also be a symptom of the general disturbance of equilibrium in this whorl.

The tendency to the reduction of the inner whorl of the androecium is of interest because, in the great majority of the Gramineae outside the Bamboos, it is the three stamens of the outer whorl which have alone survived. In these abnormal flowers of *Cephalostachyum virgatum* we are apparently witnessing the initial stages of a loss similar to that which has been completed and stereotyped in the three-stamened Grasses.

(iv) **Gynoecium Variation.**

(a) *Variation in the Ovary.*

A certain number of the flowers in the material from the Calcutta Botanic Garden seem to be sterile—they contain stamens and a gynoecium, but no ovule. Many of the flowers happen, however, to be very young, and it is possible, though not perhaps very likely, that in some of these apparently infertile ovaries an ovule might be differentiated later. In the sterile flowers the ovary wall may or may not close completely, and a lobe of vegetative tissue, which might at a cursory glance be mistaken for an ovule,

projects into the ovary cavity (cf. Fig. 2, B, p. 52; Fig. 5, B, p. 63; Fig. 6, A, p. 65; Fig. 9, B₁, p. 71). This abnormality seems to be particularly frequent in the case of those gynoecia which are associated with a stamen-lodicule.

We meet with occasional flowers, on the other hand, in which there are two ovules instead of the single ovule characteristic of the Gramineae. I have found five flowers in which the ovary is biovulate; Fig. 8, A, illustrates this condition.

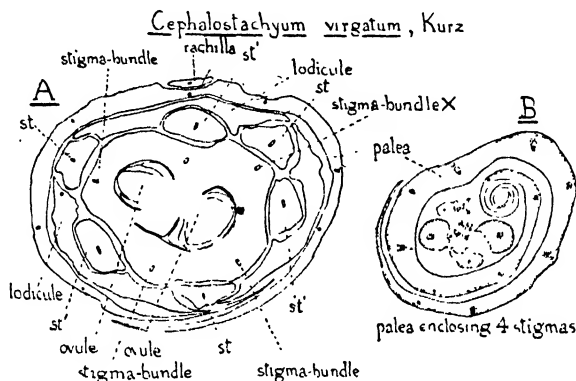


FIG. 8. *Cephalostachyum virgatum*, Kurz, Royal Botanic Garden, Calcutta. A and B, transverse sections at two levels through ovules (A) and through stigmas (B) of a flower with biovulate gynoecium ($\times 47$). Above this flower the rachilla bore an abortive flower. The back bundle of the gynoecium branches at a level above A, and the branch nearer the median plane dies out, while that to the right fuses with the stigma bundle marked X, which passes into one of the larger stigmas.

(b) *Variation in the Vascular Scheme and the Stigmas.*

Cephalostachyum virgatum is described as having two or three stigmas (3), but the material from the Calcutta Garden does not conform to this description. I have been able to observe the stigmas with certainty in thirty-one flowers. I only found as few as two in a single flower, and here one of the two stigmas contained two bundles and probably originated through failure of two of the three stigmas to separate.

In fourteen flowers I found three stigmas. A number of examples of gynoecia with three stigmas are illustrated here (e.g. Fig. 1, E₂–E₅, p. 50). The three stigmas are supplied by the three gynoecium bundles (marked *a*, *b*, *c*, in Fig. 1, D and G, p. 50) which are opposite to the outer stamens; this is very clearly seen in Fig. 1, E₃, where the three outer stamens, being taller than the others, can still just be recognized, with the stigma bundles immediately outside them. There is a fourth strand in these gynoecia, which lies at the back and supplies the ovule, when present.

In one flower I observed three stigmas of which one was larger than the other two, and for some distance contained two bundles. This flower

serves as a transition to the next group, which consists of thirteen flowers, each of which has four stigmas. Some but not all of these flowers with four stigmas are sterile; fertile flowers with four stigmas are drawn in Fig. 2, I, p. 52 (one ovule), and Fig. 8, A and B, p. 70 (two ovules). The vascular system of the flowers with four stigmas is of some interest; an example

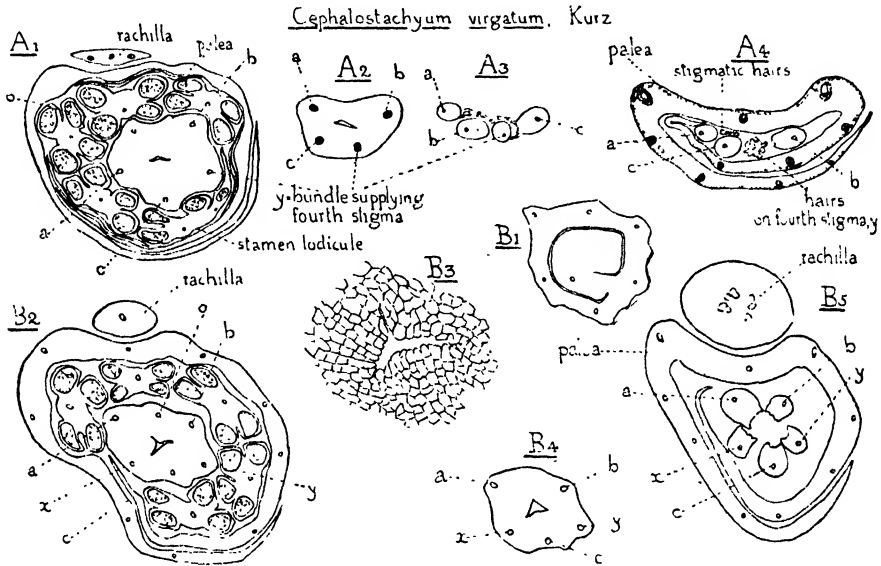


FIG. 9. *Cephalostachyum virgatum*, Kurz, Royal Botanic Garden, Calcutta. *Stigma variation.* A₁-A₄, series of transverse sections ($\times 47$) through a flower with four stigmas. A₁, through upper part of ovary at the level at which its cavity is a mere chink; there is no ovule. The back bundle marked *o*, which occupies the position of an ovule strand, dies out after giving branches to the bundles on either side. The bundle *y* supplies the fourth and smallest stigma. A₂, the gynoecium only, at a level above A₁, at which *o* has disappeared; the bundles have acquired sclerized sheaths; A₃, four stigmas; A₄, palea and stigmas at a higher level; the fourth stigma, *y*, scarcely reaches to this level, but the tuft of stigmatic hairs at its tip can be seen. B₁-B₅, transverse sections from a flower of which a section lower down is shown in Fig. 7, E, p. 66 ($\times 77$), except B₃ which is $\times 318$. B₁, gynoecium only; no ovule, but an internal lobe, which turns upwards and bifurcates, is given off into the cavity of the ovary. B₂, near top of ovary, showing the six bundles, *a*, *b*, *c*, *o*, *x*, *y*. B₃, the chink and surrounding cells in the centre of the gynoecium in B₂ (enlarged to show meristematic closure of cavity). B₄, at a higher level; the bundle *o* has disappeared by fusion with its neighbour, *a*; B₅, above the ovary to show five stigmas supplied by the five bundles in B₄. The bundle *a*, which was supplemented by the back bundle *o*, supplies the largest stigma. The enlargement of the rachilla in B₅, as compared with B₂, is in preparation for the giving off of an abortive flower, above which the rachilla continues for a short distance, and gives rise to another flowering glume (subtending no flower) before it finally dies out.

is drawn in Fig. 9, A₁. Here the back bundle *o* disappears after giving branches to the bundles on either hand, while three stigmas are supplied by the bundles *a*, *b*, *c*, and a fourth by a bundle which I have called *y*, which lies midway between *b* and *c*. Another example of a flower with four stigmas is shown in Fig. 6, C, p. 65.

I have, moreover, found two flowers in which no less than five stigmas are developed. One is drawn in Fig. 9, B₁-B₅. The gynoecium is sterile, the ovary cavity being filled up by a lobe of tissue (B₁)

which turns upwards and bifurcates. (In the only other flower in which five stigmas were observed, there appeared to be two ovules, but as the preservation was poor, I cannot be absolutely certain on this point.) High in the ovary (Fig. 9, B₂) there are six bundles—the back bundle *o*; the three bundles *a*, *b*, *c*, corresponding in position to those which supply the three stigmas in more normal flowers; and two other bundles *x* and *y*, lying respectively between *a* and *c*, and between *b* and *c*. Near the top of the ovary the bundle *o* disappears by fusion with its neighbour, *a* (B₄), and the bundles *a*, *b*, *c*, *x*, and *y*, enter five stigmas; that supplied by *a* is the largest, no doubt because *a* was supplemented by fusion with *o*.

(c) *The Significance of the Occurrence of Flowers with four or five Stigmas in Relation to the Morphology of the Gynoecium in the Grasses.*

The vascular scheme of the normal flowers of *Cephalostachyum virgatum* is one which is found widely among the Bamboos (1). This scheme is characterized by a back strand (*o*, opposite the back inner stamen) which supplies the ovule, and three strands (*a*, *b*, *c*, opposite the outer stamens) which supply the stigmas. In the material from the Calcutta Botanic Garden, however, I have found a number of flowers (described in the preceding Section of this paper) in which additional bundles (*x* and *y*) occur opposite to one or both of the two front inner stamens. If both strands are developed, we have six bundles. In the first of these 'Studies' (1, pp. 466–7, and Fig. 4, A₂, B₁, and D₂, p. 454) I have described the occurrence of corresponding bundles in the genus *Gigantochloa*, and I have discussed their bearing upon the gynoeceum theory of E. R. Saunders (7), according to which the Gramineae possess—in an extremely reduced form—two alternating whorls each consisting of three carpels. The conclusion which I drew was that although the presence of these bundles was precisely what might have been expected on Saunders's view, the fact of their existence was not in itself sufficient to afford actual proof of the validity of the theory for the case of the Gramineae. I think, however, that the variations in *Cephalostachyum virgatum* here described carry us a step nearer to a proof, for, whereas in *Gigantochloa* the extra bundles die out without influencing the external form of the gynoeceum, in *Cephalostachyum virgatum* we have examples in which the front lateral bundles of the inner whorl (those usually absent in the Gramineae) each supply an additional stigma (Fig. 9, B₂, B₄, and B₅, p. 71), so that five of the six hypothetical carpels are represented by stigmas, while the sixth (posterior median) is the only one which is (actually or potentially) fertile.

4. SUMMARY.

In this paper the results are recorded of the examination by means of serial sections of seventy flowers of *Cephalostachyum virgatum*, Kurz (Bambuseae), cultivated in the Royal Botanic Garden, Sibpur, Calcutta. These flowers show certain abnormalities which seem to have a bearing on the vexed question of the interpretation of the floral morphology of the Gramineae. The main points which emerge from their study are the following:

1. In a considerable proportion of the flowers examined, organs occur transitional in character between stamens and lodicules. These seem to originate either (*a*) by *lodicular modification of a single stamen*, generally a member of the outer whorl; or (*b*) by fusion of *one lodicule and one stamen*; or (*c*) by fusion of *two lodicules and one stamen*. In the material studied (*a*) is common, (*b*) is less common, and (*c*) is very rare. The existence of these stamen-lodicules is regarded as favouring the interpretation of the lodicules of the Grass flower as an inner whorl of perianth members.

The evidence afforded by these stamen-lodicules in relation to the general question of the morphology of the Angiospermic androecium will be considered in a later paper.

2. In addition to the free stamen-lodicule, a related structure of a more problematic nature occurs in certain flowers. This structure might be regarded as an outgrowth of the gynoeceum wall in which pollen-sacs are embedded, but I think there is better evidence for interpreting it as *a stamen-lodicule fused with the gynoeceum*, though I recognize that there are some difficulties in extending this interpretation to all the cases observed.

3. There is a strong tendency to *reduction* in the number of the stamens by *sacrifice of members of the inner whorl of the androecium*—as a rule, the front laterals. The grades of reduction observed are the following, but it is impossible to say whether they actually form a sequence: (*a*) fusion of two pollen-sacs for part of their course, (*b*) permanent reduction in the number of pollen-sacs, (*c*) loss of anther, (*d*) loss of filament and hence complete elimination.

This tendency to degradation and loss in the inner whorl of the androecium is of significance because it is the whorl which is absent in the three-stamened Gramineae.

4. The normal ovary has four bundles—the back bundle supplying the ovule, and three others entering the three stigmas. But among the flowers here described, I have met with a six-bundled type producing five stigmas (Fig. 9, B₅, p. 71), and also transitional forms with four stigmas. The occurrence of such abnormalities is just what might have been predicted on Saunders's theory (7), according to which the gynoeceum of the Gramineae

consists of two whorls, each of three carpels, the back member of the inner whorl alone being fertile; on this theory the five stigmas would represent five sterile carpels. The discovery of these unusual forms does not afford absolute proof of the validity of Saunders's interpretation of the Grass gynoeceum, but it may be held to add somewhat to its probability.

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Notes on the Anatomy of some New Zealand Species of *Dacrydium*.

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With Plate VII and four Figures in the Text.

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THE present work, begun by one of us in 1918 at the Cambridge Botany School, has, after a gap of several years, been continued at Lucknow as a joint investigation. For the material originally examined we wish to express our sincerest thanks to Professor A. C. Seward, F.R.S. This consisted of vegetative shoots and female strobili of three species, *D. Bidwillii*, *D. Colensoi*, and *D. laxifolium*, as well as herbarium material of *D. Kirkii*. More recently, Mr. C. E. Foweraker, of Canterbury College, Christchurch (N.Z.), sent us some well-fixed ovuliferous shoots of *D. biforme*, for which also we are very thankful.

Although the quantity of material was inadequate for anything like a full investigation, it has yielded some interesting facts which are here recorded; in some respects they differ from the observations of previous authors, and in others they extend our knowledge of a genus deserving of closer study.

DESCRIPTIVE.

1. *Dacrydium Bidwillii*, Hook. f.¹

We will first summarize in a few words our main observations on this species, and then pass on to a fuller description.

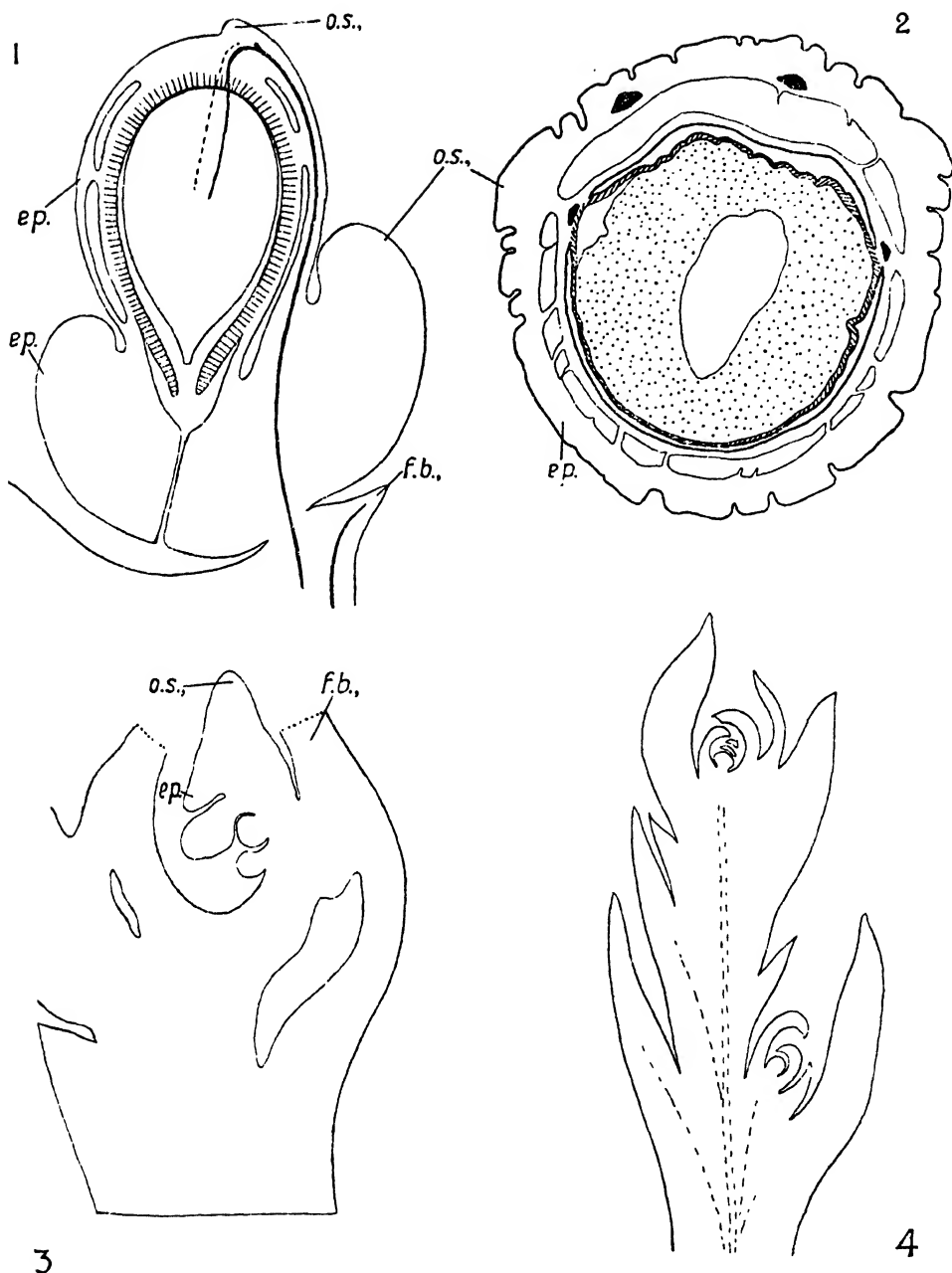
Owing to its completely inverted ovules and to the fact that the epimatium, extending up to the micropyle, conceals the whole of the integument, *D. Bidwillii* has generally been regarded as forming a transition to the genus *Podocarpus*. This view of Pilger's (loc. cit., p. 17) has been adopted by Sinnott, who has given the best and most recent account of the ovules. Our observations differ somewhat from those of Sinnott; but the differences are at least partly due to the fact that the ovules examined by us were older than his. Firstly, in all the ripe ovules (seeds) seen by us there is a large fleshy aril-like swelling at the micropylar end, which he does not mention (see our Text-fig. 1 and Pl. VII, Figs. 1-9). Secondly, we find that the epimatium, rather than being free from the integument down to the chalaza (an important distinction from *Podocarpus* where the two are more or less fused together), is united to it for more than half the ovule length. Thirdly, the integument is supplied by two (rarely three) vascular bundles, which have not so far been noticed. These bundles descend from the chalaza, right and left of the ovule, till they reach half-way down to the micropyle. They no doubt correspond to the system of 'descending' strands in the *Podocarpus* ovule.

With the exception of the 'aril', which is a rather peculiar feature, the above facts, taken together, appear to point to the genus *Podocarpus* rather than to *Dacrydium*. In fact, we believe that the plant is really a species of *Podocarpus*, wrongly referred to the genus *Dacrydium*.

In our material the megastrobili have mostly two fertile bracts, each with a completely inverted ovule in its axil. Between the ovules the axis ends in a bud such as may often be seen in *Podocarpus* (Pl. VII, Figs. 1, 1a).

(a) The 'aril' stands out owing to its white colour and large size, exceeding that of the ovule itself. It lies between the fertile bract and the

¹ Hook. f. ex Kirk in Trans. N. Z. Inst., x, 1878, 388; Kirk: Forest Flora of New Zealand, 1889, lvii, tom. 37 (Fig. 3, shoot with dimorphic leaves; Figs. 1', 2', 3', fruits with fleshy 'involucral cup'); Pilger in Engler's Pflanzenreich, 1903, p. 46, Fig. 4; Cheeseman: Manual N. Z. Flora, 1906, pp. 653-4; Sinnott, E. W.: Annals of Botany, 1913, vol. xxvii, p. 39; Sahni, B.: Nature, 1918, vol. cii, p. 299.



TEXT-FIG. 1. '*Dacrydium*' *Bidwillii*. Diagrammatic longitudinal section of an ovule after fertilization. One of the 'ascending' and one of the 'descending' strands are shown as a continuous line. The broken line represents the other descending; the corresponding ascending strand is omitted. TEXT-FIG. 2. '*D.*' *Bidwillii*. Diagram prepared from camera-lucida sketches of two microtome sections of the same ovule taken midway between the chalaza and the micropyle. In the region of the descending strands the nucellus (shaded) is clearly seen fused with the integument, which in turn is fused to the inner layer of the epimatium. The large secretory canals lie in the epimatium and ovuliferous scale. Compare with the photograph in Pl. VII, Fig. 10. TEXT-FIG. 3. *D. Colensoi*. Longitudinal section of part of a very young megastrobilus. TEXT-FIG. 4. *D. laxifolium*. Longitudinal section of a very young megastrobilus.

vule, forming a soft cushion in which the ovule is partly embedded (Pl. VII, figs. 1-7). When the fruit is ripe the 'aril' readily comes off with the vule (Pl. VII, Fig. 2), leaving the fertile bract on the axis. It appears that the seed is dispersed through the agency of birds, as in *Taxus*. The delicate succulent 'aril' being easily digested, the hard and smooth seed, which is longitudinally ribbed, would pass out with the excreta unharmed.

We are not the first to describe this organ which we have here (more conveniently than correctly) called an 'aril'. Kirk refers (loc. cit., p. 57) to an 'involucral cup', sometimes becoming 'fleshy, white and smooth'. Pilger (loc. cit., p. 17) describes a ring-shaped swelling round the point of attachment of the ovule, but his figures do not show it. Two of our ovules are devoid of an 'aril', and these appear to be abortive, suggesting that the development of this structure depends upon fertilization. Sinnott (loc. cit., p. 49) mentions 'a thickened corky ring at the micropylar end', but there is no suggestion of fleshiness.

Pl. VII, Fig. 4 shows the 'aril' from the lower end. On the left is the scar where it was attached to the axis; this is the point of entry of the 'ascending' bundles. On the right is seen a hole, sometimes, as in this case, slit-like; this leads along a narrow passage through the 'aril' to the micropyle, which is itself hidden from view (see Fig. 7). Pl. VII, Figs. 7-9 are serial transverse sections from below upwards, taken in the region of the 'aril'; the ventral¹ side being in each case towards the top of the figure. In Fig. 7 the section passes through the micropylar tube, seen lying in the slit-like passage through the 'aril'. Fig. 8, showing the 'aril' in two lobes, is specially instructive. The part containing the two bundles is the ovuliferous scale, of which the right and left margins can also be made out on the surface of the seed, and of which the distal end is seen as a knob-like projection, the 'apical knob' of Sinnott (see Pl. VII, Figs. 1-3, 5, 6). The ventral lobe of the 'aril' is seen as a fleshy outgrowth of the ovuliferous scale. The dorsal lobe is a similar enlargement of the epimatium, itself probably a part of the ovuliferous scale arching over the ovule. Comparison with a longitudinal section (Text-fig. 1 and Pl. VII, Figs. 5, 6) shows that the 'aril' is a swelling partly of the epimatial margin where it surrounds the micropyle, partly of the base of the ovuliferous scale. It is therefore comparable to part of the outer flesh of the *Podocarpus* seed; the only difference is that the fleshiness is confined to one end of the seed and shows an enormous development.

(b) *Integument, Epimatium, &c.* The longitudinal sections in Pl. VII, Figs. 5, 6, clearly show that at least along the plane of section the epimatium is fused to the integument on both sides for more than half the ovule length, as in *Podocarpus* (see also Pl. VII, Fig. 10, and Text-fig. 2). The free portion

¹ That is, the side of the ovule which is adnate to the scale.

of the integument is marked by its greater thickness and the presence of sclerotic cells. The rest of the integument is quite thin and fused with the inner layer of the epimatium, which is separated from its outer layer by wide resin-spaces. The nucellar apex forms a long tapering beak which engages with the micropylar canal. The rest of the nucellus is at this late stage reduced to a brittle papery lining, and it is difficult to say from our longitudinal sections whether it is free from the integument down to the chalaza (as described by Sinnott in the young ovule), or whether it becomes more or less fused with it during growth. Our sections show it closely applied to the integument for a short distance from the chalaza (as in Text-fig. 1). Whether it is there fused to it we cannot say. The transverse sections (Text-fig. 2 and Pl. VII, Fig. 10) are more instructive, and show beyond doubt that along the right-left plane the nucellus is fused to the integument for about half the length, but is free elsewhere.

The ovules are too far advanced to show anything of the male gametophyte. The multinucleate tissue of the female prothallus already showed a small embryo at the end of a long and tortuous suspensor. There is a thick megaspore membrane having a prismatic structure.

(c) *Vascular Supply*. Sinnott has described the vascular supply very clearly, and our observations agree with his description so far as it goes. But we find that the two ovular bundles, shown by him as curving over and ending at the chalaza (see his Text-fig. 5 A), are in fact continued downwards right and left at least as far as the equator. Near the chalaza, where the bundles curve over, each gives off a few tracheides which apparently belong to the apical knob. These tracheides may, by analogy with *Podocarpus*, be regarded as the ends of the ovuliferous scale bundles, of which the descending strands are branches. Text-fig. 2 is from a camera-lucida sketch combining two microtome sections of the same ovule cut near the middle. It not only shows the two descending strands, but also the fact that along the plane of these strands even the nucellus is fused to the integument. One of these two sections is photographed in Pl. VII, Fig. 10.

In Text-fig. 1 we have shown, somewhat diagrammatically, the more important points in the ripe ovule as described above. It will be noticed that, except for the 'aril' (which may be regarded as a partial sarcotesta), the structure is almost exactly that of a *Podocarpus* ovule (compare, e.g., Sinnott's figure of *P. ferrugineus*, loc. cit., p. 46).

(d) *Affinities*. As already stated, we regard this plant as a species of *Podocarpus* and not of *Dacrydium*. This view appears fully justified if we consider *en masse* the characters of the female organs, on which alone the generic distinction is based. Thus, as in *Podocarpus*, and unlike the usual condition in *Dacrydium*, the ripe seed is completely inverted; the epimatium completely covers the integument, and is more or less fused to it. Moreover, the epimatium is at least partly fleshy ('aril'), whereas in *Dacrydium*,

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so far as known, it is always dry. In no other character does the plant show any essential difference from *Podocarpus*. When in nearly all respects it falls into line with species of *Podocarpus* we see no reason to retain it under *Dacrydium* as an aberrant member of that genus. We therefore suggest that it should henceforth be called *Podocarpus Bidwillii* (Hook. f.);¹ and, to give expression to its peculiarity—the fact that the seed is partly dry and partly fleshy—we would place it in a new and distinct section of the genus, namely §*Bidwillii*, closely allied to §*Stachycarpus*.

2. *Dacrydium biforme*, Hook. sp.²

Vegetative Characters. As Cheeseman says this plant closely resembles 'D.' *Bidwillii* in some respects, the most notable being the completely inverted ovule, but the general habit of the two species appears to be very distinct. A curious point is that the strongly keeled adpressed leaves have an incurved acuminate tip which often lodges in a pocket on the under side of the leaf next above (Pl. VII, Fig. 11).

Megastrobilus. We had at our disposal only three or four strobili, all with ovules at a stage just before fertilization. In our material only one or two ovules appear to become ripe in each strobilus. The appearance of the ovules and adjacent organs is strikingly similar to that of 'D.' *Bidwillii* before the development of the 'aril'. There is the same longitudinally striated, somewhat compressed, ovule, with an 'apical knob' near the chalazal end. The vascular supply is identical in the two cases: here also a pair of inverted bundles ascend along the ovuliferous scale, and just before ending in the apical knob each bundle gives off a descending branch which can be followed down about half-way to the micropyle. The epimatium, however, does not quite reach the tip of the micropylar canal (Pl. VII, Fig. 11), and the integument appears to hang quite free in the bell-shaped cavity of the epimatium (as Sinnott describes for the young stage of 'D.' *Bidwillii*). The nucellus appears to be fused to the integument along the plane of the two descending strands, being free from it elsewhere.

Archegonia. There is a pair of large archegonia (742 μ long, 151 μ wide), each with a well-developed layer of jacket-cells which are distinguished by their dense contents and relatively huge nuclei. The peripheral cytoplasm of the egg is markedly vacuolate; the large spherical egg-nucleus

¹ Not to be confused with *P. Bidwillii*, Hoibr. which is a synonym of *P. spinulosus* (Smith); see Pilger, loc. cit., p. 76.

² Hooker, W. J.: Icon. Plant., 1843, tom. 544 (earliest description of the species, under the name *Podocarpus* (?) *biformis*; figures two kinds of leaves on the same shoot, but the flowers were then unknown); Hooker, J. D.: Flor. Nov. Zeland., i, 1853, p. 234 *pro parte D. Colensoi*; Hooker, J. D.: Handbook N. Z. Flora, 1867, p. 259 (wrongly under the name *D. Colensoi*); Kirk, T.: Forest Flora of N. Z., 1889, p. 189, tom. 96 (figures young plant with dimorphic leaves under the name *D. Colensoi*, Hook.); Pilger, R.: in Engler's Pflanzenreich, 1903, Heft 18, p. 45; Cheeseman: Manual N. Z. Flora, 1906, p. 653.

(106 μ in diameter) lies in the broad upper part of the cell, with the ventral canal-nucleus (45 μ) above and, so far as we have seen, always a little to one side of the egg-nucleus. The stage figured in Pl. VII, Fig. 12, is just before fertilization. The apex of the egg in the archegonium on the right has been hollowed by the tip of the advancing pollen tube, which contains a number of nuclei. The quantity of material was inadequate for a study of the male gametophyte. The megaspore membrane, as in '*D.*' *Bidwillii*, is thick (about 5 μ), and shows a cross-striated appearance.

Affinities. Any discussion of the affinities of *D. biforme* must take into account the resemblances, some of them striking, which the species shows to '*D.*' *Bidwillii*, a species we have referred to the genus *Podocarpus*. The question naturally arises whether *D. biforme* should not be considered in the same light. The plant was, in fact, originally described by W. J. Hooker under the name *Podocarpus* (?) *biforme*, though the flowers were not known to him at the time. The ripe ovules have never, so far as we know, been examined anatomically. Till this is done the question of generic position must remain open.

3. *D. Kirkii*, F. Muell.

Another interesting New Zealand species is *D. Kirkii*, which closely resembles *D. biforme* and '*D.*' *Bidwillii* in the shape and position of the ovules. Of this we have only been able to cut ovules of an advanced age from herbarium material. The megaspore membrane is exactly as in the two species previously described.

All three species appear to form a compact little group, worthy of closer attention by those favourably situated with regard to material.

4. *Dacrydium Colensoi*, W. J. Hooker.¹

Megastrobilus. In the youngest stage (Text-fig. 3) the ovule is either horizontal or faces obliquely downwards;² it lies protected in a socket between the fertile bract and the vegetative axis, which usually terminates between the ovules. The ovuliferous scale is at this time a very prominent feature projecting far beyond the ovule, with the epimatium as only a small ridge. The upper lip of the integument is much thicker than the lower.

A little later (Pl. VII, Fig. 14) the ovule begins to erect itself, and the epimatium considerably enlarges, now forming a hood which not only reaches the micropyle but also envelops the ovule at the sides. A few stomata are seen on the ovuliferous scale, but none on the epimatium. As the ovule is

¹ Hooker, W. J.: Icon. Plant., 1843, tom. 548; Hooker, J. D.: Flor. Nov. Zel., i, 1853, p. 234; Kirk, T.: For. Flor. N. Z., 1889, p. 165, tom. 85 (under *D. westlandicum*, Kirk; Fig. 11, ovule at about pollination time; Figs. 12-14, fruit); Cheeseman, T. F.: Manual N. Z. Flora, 1906, pp. 655-6; Stiles, W.: A Note on the Gametophytes of *Dacrydium*, New Phytologist, 1911, vol. x, p. 344.

² Goebel, K.: Organography of Plants (Engl. ed.), Part II, 1905, p. 519, Fig. 348, 1, II.

seated on the scale with a broad base, the term ovular stalk for the scale is not here so apt as in *Podocarpus*, where it has been compared to the funicle of an anatropous ovule. Its homology with the ovuliferous scale of other Conifers is also more apparent here than in *Podocarpus*. But the surest clue is afforded by the inverted bundles which are such a constant feature of the allied genera *Dacrydium*, *Podocarpus*, and *Acmopyle*.¹

In the oldest stage examined (Pl. VII, Fig. 15, 15a) the ovule is seen projecting far beyond the epimatium. Unlike the species previously described, the ovule of *D. Colensoi* has a thick integument differentiated into three well-defined layers. The nucellus, at first 'superior', later becomes sub-inferior, the shoulder being considerably higher on the epimatial side than on the other (Pl. VII, Fig. 13). The preservation of the material is too poor to show the structure of the female prothallus.

Vascular Supply. The trace of the fertile bract, like that of the foliage leaf, ends in a mass of transfusion tracheides. In the comparatively young stage (Pl. VII, Fig. 14) the two inverted bundles supplying the ovuliferous scale penetrate well beyond the chalaza in the direction of the apical knob, though they do not actually enter the latter. In the older stage (Pl. VII, Fig. 13) they fuse together at the chalaza into a pad of short, broad tracheides. From this pad two well-developed bundles are given off to supply the ovule. They pass into the inner flesh through two chalazal foramina in the stony layer. Each of these bundles has a concentric structure, consisting of tracheides surrounding a core of thin-walled cells (? phloem). As the bundle ascends along the inner flesh it expands somewhat like a funnel till it reaches the shoulder. From here it is continued as a slender row of tracheides well beyond the shoulder into the well-developed inner fleshy layer. No trace of a nucellar supply has been found.

5. *Dacrydium laxifolium*, Hook. f.²

Habit and Vegetative Structure. This diminutive plant is probably the smallest known Conifer, sometimes fruiting when barely 2 in. high. It forms thick mats on the subalpine moors of New Zealand, at levels between

¹ Stiles, W.: The Podocarpeae, Ann. Bot., 1912, vol. xxvi, pp. 470-77; Gibbs, L. S.: Development of the Female Strobilus in *Podocarpus*, Ann. Bot., 1912, vol. xxvi, pp. 528, 532, 536, 540, 542, &c.; Sinnott, E. W.: loc. cit., p. 42; Brooks, F. T., and Stiles, W.: The Structure of *Podocarpus spinulosus*, Ann. Bot., 1910, vol. xxiv, p. 312; Sahni, B.: On the Structure and Affinities of *Acmopyle Pancheri*, Phil. Trans., 1920, vol. ccx, p. 268.

² Hooker, J. D.: Lond. Journ. of Bot., iv, 1845, p. 143; Hooker, W. J.: Icon. Plant., 1852, tom. 815 (shows no trace of a swollen receptacle); Hooker, J. D.: Flor. Nov. Zel., i, 1853, p. 234 ('fruit scarlet, terminal or lateral'); Hooker, J. D.: Handbook N.Z. Flora, 1867, p. 259 ('nuts small, erect, in red fleshy cups'); Carrière, Conif., ii, 1867, p. 692 (does not mention a fleshy receptacle); Kirk, T.: For. Flor. N.Z., 1889, p. 169, tom. 87 (shows fruits with dry and with fleshy receptacles, and young ovules horizontally placed; 'fruit, a cylindrical nut with a membranous cup at its base, seated on a coriaceous or pulpy crimson receptacle', p. 170); Pilger, R.: in Engler's Pflanzenreich, 1903, Heft 18, p. 50; Thomson, R. B.: The Megaspore Membrane of the Gymno-

2,500 and 4,000 ft. above the sea. Kirk figures three kinds of leaves: (*a*) those on the young plant, subulate and spreading, $\frac{1}{4}$ to $\frac{1}{2}$ in. long, and those on the mature branches, which are either (*b*) appressed and scale-like, or (*c*) lax and spreading, about $\frac{1}{12}$ in. long. Our material shows only the last-mentioned type, which gives to the plant a habit very distinct from that of other Podocarps (Pl. VII, Fig. 16). The plant is also peculiar (and, so far as we know, unique among the family) in the absence of any resin-canals or other secretory sacs in the stem, leaves, and ovules. The growth of the plant must be extremely slow: a slender axis with a xylem cylinder barely 0.35 mm. in radial thickness already showed half a dozen growth-rings (Pl. VII, Fig. 17). The medullary rays are uniseriate, 1-7 cells high (mostly 1-3). A striking peculiarity is the total absence of xylem parenchyma and the abundance of tangential pits throughout the wood. The tracheides being narrow the radial pits are nearly always uniseriate, usually circular and separate, sometimes contiguous and flattened above and below. When biseriate they are alternate and hexagonal in the few cases observed. This araucarioid tendency, recorded by Stiles in *Saxegothaea*,¹ has also been noticed in *Dacrydium* by Gothan.² The pits in the field are 2-5 or 6 (usually 3 in a vertical row, the field being narrow).

The leaf has a flat upper and convex lower surface with the central tissues remarkably loose (Pl. VII, Fig. 18). Stomata of the usual coniferous type, slightly sunk below a thick cuticle, occur on all sides. The two bands of transfusion tissue are well developed, frequently joining each other in an arc over the protoxylem; the tracheides are either of the usual type (with bordered pits on all the walls) or show band-like thickenings in addition, as in *Acmopyle*³ and other Conifers.

Megastrobilus. *D. laxifolium* is also notable owing to the frequent (though apparently not constant) presence of a fleshy receptacle, formed of the enlarged bracts—a feature more characteristic of *Podocarpus* than of *Dacrydium* (Pl. VII, Fig. 19). The single dry seed, with a peculiar recurved micropyle, is seated on a tumid receptacle recalling the way in which the dry carpel of *Anacardium occidentale* is seated on the much-enlarged and succulent thalamus. An interesting comparison may also be made with the New Zealand species *Exocarpus Bidwillii*, Hook. fil. (Kirk: For. Fl. of N.Z., Pl. 52), a member of the Santalaceae, in which the peduncle of the flower

sperms, Univ. of Toronto Studies, Biol. Series No. 4 (describes and figures a thick and double megaspore membrane); Cheeseman, T. F.: Man. N.Z. Flora, 1906, p. 657 ('receptacle sometimes dry, sometimes swollen and succulent'); Stiles, W.: A Note on the Gametophytes of *Dacrydium*. New Phytologist, 1911, vol. x, p. 345, Fig. 1; Dallimore and Jackson, 1923, p. 30 (local name Mountain Rimu; seed 'borne on a dry or occasionally succulent and swollen receptacle').

¹ Stiles, W.: The Anatomy of *Saxegothaea conspicua*, New Phytologist, 1908, vol. vii, p. 209.

² Gothan, W.: Zur Anatomie lebender u. foss. Gymnospermenhölzer, Abh. K. preuss. geol. Anst., N. F., Heft 44, 1905, p. 57.

³ Sahni, B.: loc. cit., p. 260, Text-fig. 4.

becomes a scarlet berry in the fruit, with the black 'nut' seated on it terminally. According to Kirk (loc. cit., p. 170) the receptacle of *D. laxifolium* has a crimson colour. Possibly, as suggested in the case of *D. Bidwillii*, the seed is distributed through the agency of birds, the attractive apparatus being the bracts in the one case, the ovuliferous scale and epimatium in the other.

The very young strobilus (Text-fig. 4), in the only specimen available, is a slender shoot with several bracts and bearing two ovules, each of them apparently terminating an axis. Pl. VII, Fig. 20 is a longitudinal section through an older strobilus, with the single erect ovule at about pollination time. The bracts are already becoming fleshy; so also the epimatium, on which, curiously enough, no trace of a knob is visible. Stomata are present on both surfaces of the epimatium. The epidermal cells of the receptacle are often produced into short papillae. The epimatium is quite free from the integument and passes completely round it, its edges overlapping on the other side (Pl. VII, Fig. 21). A striking feature is the strongly curved micropyle, with a much extended hook-shaped upper lip. The female prothallus, with free nuclei, lies deep down in the base of the nucellus as observed by Stiles. The middle region of the nucellus is remarkable for its very thick cuticle, which gradually thins towards the base and apex. The cells in the distal part of the nucellus are somewhat elongated and have slightly thickened walls which take up the gentian violet stain in preference to orange G.

In the oldest stage examined (Pl. VII, Fig. 19) the integument is clearly differentiated into three layers; the stony layer consists of cells with thickenings unusually like those of xylem tracheides.¹ The epimatium now forms a relatively small cup at the base, partly concealed by the swollen receptacle.

Vascular Supply. The vascular supply is similar to that in *D. Colensoi*. After the sterile bracts have been supplied, three strands are left in the receptacle. One of these enters the fertile bract, where it spreads out tangentially before ending in a mass of rather scattered transfusion tracheides; the other two,² after the usual rotation, pass to the chalaza and even project a little beyond the latter, as if intended for an ovuliferous

¹ They are, however, at an early stage in sclerization, as one can see by the presence of large nuclei and dense protoplasmic contents. The appearance of these tracheide-like cells suggests that the stony layer (not only of this but of other seeds as well) may, in addition to its protective function, be acting as a water-reservoir, to be drawn upon possibly during the intraseminal development of the embryo after the seed is shed.

² Such paired strands are sometimes seen even in the axils of sterile bract-traces. But they end blindly, like similar ones described in species of *Podocarpus* (Brooks and Stiles, 1910, Ann. Bot., vol. xxiv, pp. 311-13) and in *Acmopyle* (Sahni, Phil. Trans., 1920, vol. ccx, p. 267). These blind-ending strands no doubt represent the vestigial vascular supply of reduced ovuliferous organs, which either do not develop at all, or, as in *Acmopyle*, are represented by mere sterile humps in the axils of the bracts.

scale indistinguishably fused with the epimatium. At the chalaza they flatten out and fuse into a pad of short tracheides, from which, as in *D. Colensoi*, a pair of slender strands penetrate into the inner fleshy layer. These bundles have been followed along this layer well beyond the shoulder, about half-way up to the micropyle.

From this brief account it appears that in several respects *D. laxifolium* stands somewhat apart from the other species of the genus.

THEORETICAL.

(a) *The Systematic Position of 'D.' Bidwillii.*

The theoretical bearing of the points brought out in these notes chiefly concerns the interrelations of the genera *Dacrydium* and *Podocarpus*. As is well known, these two genera are not sharply distinguished; the only important distinctions are based on the characters of the female organs, but even there exceptions are frequent. Thus:

<i>Dacrydium</i> .	<i>Podocarpus</i> (inc. 'D.' <i>Bidwillii</i>).
a. No fleshy receptacle (exc. <i>D. laxifolium</i> , <i>D. cupressinum</i> , <i>D. taxoides</i> , <i>D. intermedium</i>).	Fleshy receptacle present (exc. <i>Stachycarpus</i> , &c.; also 'D.' <i>Bidwillii</i>).
b. Ripe seed dry.	Ripe seed usually with a succulent outer coat (partly dry, partly fleshy in 'D.' <i>Bidwillii</i>).
c. Ripe seed not completely inverted (exc. <i>D. Kirkii</i> and <i>D. biforme</i> , both possibly spp. of <i>Podocarpus</i> ?).	Ripe seed always completely inverted.
d. Epimatium in ripe seed entirely free from integument down to the chalaza (condition in <i>D. Kirkii</i> and <i>D. biforme</i> not known).	Epimatium in ripe seed more or less fused to integument.
e. Epimatium in ripe seed not concealing the micropyle.	Epimatium in ripe seed concealing the micropyle.
f. Nucellus at pollination stage free down to the chalaza (exc. <i>D. Colensoi</i> , <i>D. cupressinum</i> , &c.).	Nucellus at pollination stage more or less fused to integument.

A reference to the accompanying table will show the peculiar position of '*Dacrydium*' *Bidwillii* if it were to be retained in the left-hand column. We need not repeat our reasons for transferring this species to *Podocarpus*. In that genus it fits in quite naturally, but among the *Dacrydiums* it can only remain as a very aberrant member, exceptional in several important respects. The position of *D. biforme* and *D. Kirkii*, both with inverted seeds, must remain doubtful till further material is examined. Perhaps they also belong to *Podocarpus*. In that case we would at least have one absolute distinction between the two genera, namely, the completely inverted seed in *Podocarpus*. In fact, it seems to us not unreasonable to simplify the dia-

gnosis of the two genera by transferring these species also to *Podocarpus* on the strength of this character, combined with their other striking resemblances with *P. Bidwillii*.

(b) *Epimatium and Ovniferous Scale.*

Some prominence has been given in this paper to the small process at the chalazal end of the ovule which Sinnott has called the 'apical knob'. We attach importance to it because it is useful in a comparison with other Conifers. In accord with the views of Miss Gibbs and others we regard it as the tip of an ovuliferous scale, the latter being defined as the part which carries the inverted bundles. Among other Conifers, it would correspond to the ridge at the back of the seed of *Acropyle*, and to the ligule of *Araucaria*. The epimatium we regard as only an extension of the scale, being sometimes small and free in the ripe seed (*Dacrydium*), sometimes co-extensive with the integument and fused with it (almost completely in *Podocarpus*, completely and indistinguishably in *Acropyle*).

(c) *Interrrelations of Dacrydium, Podocarpus, and Acropyle.*

It may not be out of place to discuss in a few words the affinities of these three genera with one another. We shall confine our remarks to the more obvious features, which also appear to represent the most important distinctions. *Podocarpus* and *Acropyle* agree, so far as known, in almost every point except the position of the seed and the degree of fusion of epimatium and integument:

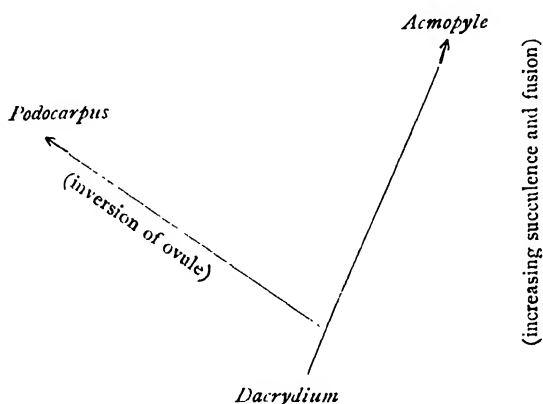
<i>Podocarpus.</i>	<i>Dacrydium.</i>	<i>Acropyle.</i>
1. Ripe seed inverted.	Ripe seed horizontal to erect.	Ripe seed semi-erect.
2. Epimatium almost completely fused to integument.	Epimatium free from integument.	Epimatium completely and indistinguishably fused to integument.

In the first character most species of *Dacrydium* resemble *Acropyle* rather than *Podocarpus* (the seed being inverted only in *D. biforme* and *D. Kirkii*, both doubtfully *Dacrydiums*, and rarely erect, as in *D. laxifolium* and *D. Fonkii*). In the second character *Dacrydium* is at one end of a series, rather nearer to *Podocarpus*.

Sinnott's study of the Podocarpeae has led him to regard this family as a reduction series, with *Podocarpus* as the most primitive member (loc. cit., p. 63). To this view we do not subscribe. If Sinnott's view were correct, the fused-up epimatium of *Podocarpus* must have gradually freed itself from the integument and lost its fleshy character to produce the condition in *Dacrydium*. The vascular supply must have become correspondingly reduced. But no adequate grounds seem to have been advanced

which make such a separation and drying up of parts appear physiologically reasonable. On the other hand, the increased development of the epimatium, with concomitant fusion to the integument, seems intelligible on the ground of better protection of the ovule. The reverse process, especially the *initial* separation of epimatium from integument, would be specially difficult to understand in *Acropyl*², for in this plant the two organs must be regarded as being completely fused together right up to the micropyle.¹

The relations may be represented diagrammatically as follows :



SUMMARY.

1. *Dacrydium Bidwillii*, Hook. f., generally regarded as an interesting transition to *Podocarpus*, chiefly owing to its inverted ovule, is really a species of that genus, and should therefore be named *Podocarpus Bidwillii* (Hook. f. *non* Hoibr.). Perhaps *D. biforme* (Hook.) and *D. Kirkii* (F. Muell.), both with similarly inverted ovules, should also be referred to *Podocarpus*, but these species need further investigation. If they also prove to be species of *Podocarpus*, the inverted position of the ripe ovule would be an absolute distinction between that genus and *Dacrydium*.

2. In *P. Bidwillii* (Hook. f.) the epimatium and ovuliferous scale, where they surround the micropyle, swell up after fertilization into a large, white and succulent aril-like organ, the rest of the seed remaining dry. In view of this peculiarity it is proposed to include the plant in a new and distinct section of the genus, § *Bidwillii*, resembling § *Stachycarpus* in the absence of a fleshy receptacle.

3. In both *P. Bidwillii* and *D. biforme* the two ovular strands at the chalaza give off two 'descending' strands, which pass downwards, right and left, to about the level of the equator. These bundles lie in the same plane as the two corresponding bundles in *P. ferrugineus*.

¹ No other explanation seems admissible, for this genus is too closely related to *Podocarpus* to be altogether devoid of an epimatium.

4. The ripe ovule of *D. Colensoi* has a thick integument, differentiated into three layers. The nucellus is fused with the integument for some little distance from the base. From a pad of tracheides at the chalaza two distinct strands pierce the integument and pass up along the inner fleshy layer. The young ovule is horizontal or slightly inverted; the ovuliferous scale being at this stage a relatively very prominent organ, with the epimatium showing as a faint ridge.

5. *D. laxifolium* is a rather aberrant member of the genus. Apart from other differences, it is peculiar among Podocarps in the absence of all trace of xylem parenchyma and of resin or mucilage canals in the stem, leaves, and ovules. The ripe seed, with a characteristic hooked micropylar tube, is seated on a fleshy receptacle which is formed, as in many species of *Podocarpus*, of swollen bracts. The vascular supply to the ovule is mainly as in *D. Colensoi*.

6. The interrelations of the genera *Dacrydium*, *Podocarpus*, and *Acmopyle* are discussed. The authors do not agree with the view that the Podocarps are a reduction series, with *Podocarpus* as its most primitive member.

It is a genuine pleasure to us to record here our deep sense of gratitude to Professor A. C. Seward, F.R.S., for his kindly interest in the work, and for his revision of the manuscript.

BOTANY DEPARTMENT,
UNIVERSITY OF LUCKNOW.
March 10, 1926.

EXPLANATION OF PLATE VII.

Illustrating Messrs. Sahni and Mitra's paper on the Anatomy of some New Zealand Species of *Dacrydium*.

(Abbreviations used: *o. s.*, ovuliferous scale; *ep.*, epimatium; *d. l.*, *v. l.*, dorsal lobe, ventral lobe of 'aril'; *v. a.*, apex of vegetative axis; *f. b.*, fertile bract.)

Fig. 1. *Dacrydium Bidwillii*. Megastrobilus with two fertile bracts, *f. b.*, each having in its axil an inverted ovule with an 'aril'. \times about 3.

Fig. 1 *a.* The same. \times 2.

Fig. 2. The same. A single detached ovule, in side view. \times 2.

Fig. 3. The same. An ovule in longitudinal section. \times 2.

Fig. 4. The same. An ovule seen from the basal end. The black dot on the left marks the point of attachment; the line on the right is the slit-like passage through the 'aril', leading to the micropyle. \times 2.

Fig. 5. The same. An ovule in longitudinal section. Shows the delicate thin-walled tissue of

the 'aril', and the large secretory canals in the *o. s.* and *ep.* The section does not pass through the slit-like passage in the 'aril'. $\times 10$.

Fig. 6. The same. An ovule in longitudinal section. * is the point of attachment, from where the pair of inverted bundles ascend into the ovuliferous scale; the narrow passage to the micropyle is clearly seen. The nucellus and female gametophyte have been pushed aside to show the fusion of integument and epimatium on the left side. $\times 10$.

Fig. 7. The same. Transverse section through the 'aril', showing the micropylar tube lying in the slit-like passage in the 'aril'. The large central space is a secretory canal; just above it are the two ascending strands. $\times 10$.

Fig. 8. The same. Transverse section at a higher level. The oval central patch is the female gametophyte, surrounded by the nucellus (black) and integument. Further explanation in text, p. 78. $\times 10$.

Fig. 9. The same. Transverse section, at a slightly lower level, of an ovule in which the 'aril' is not so well developed as in Fig. 9. Nucellus (perforated centrally by a pollen-tube) surrounded by integument. The innermost zone of the epimatium and ovuliferous scale contains a ring of large secretory canals. $\times 10$.

Fig. 10. The same. Part of a microtome section across an ovule at about the equatorial level. The integument is fused with the epimatium. The ribbed surface of the ovule is covered with a very thick cuticle. The upper arrow, lying in a secretory sac, points to one of the 'ascending' strands, the lower to a 'descending' strand. In the latter region the female prothallus, with its thick limiting membrane, has shrunk away, and the nucellus is seen fused with the integument. \times about 60.

Fig. 11. *D. biforme*. Longitudinal section through a biovulate strobilus. The tips of some of the bracts (*) lie in depressions on the abaxial side of the bracts above them. There is a close general resemblance with '*D. Bidwillii*', but an 'aril' is not present. The fertile bracts are much thicker than the sterile. \times about 14.

Fig. 12. The same. Longitudinal section through an ovule just before fertilization. Nucellar beak invaded by pollen-tube, and with a germinated pollen grain at its tip. Female prothallus with thick megaspore membrane (thinning at apex) and two archegonia. The apex of the egg-cell on the right is excavated, with the tip of a pollen-tube lying in the cavity. The large circular nucleus is that of the egg, the smaller one above and to one side is the ventral canal nucleus. \times about 56.

Fig. 13. *D. Colensoi*. Longitudinal section of a strobilus, and one abortive ovule and (?) a fertile one in a semi-erect position, far exceeding the epimatium. Nucellus obliquely fused to the thick integument; vascular pad at chalazal end; *f. b.* with large secretory canal. \times about 14.

Fig. 14. The same. Longitudinal section of young megastrobilus. Both the ovules are abortive, and have been cut slightly tangentially. Traces of the epimatium are seen on the lower side also, showing that it nearly surrounds the integument at this stage. \times about 14.

Fig. 15. The same. Ovuliferous shoots showing the oblique cup-like epimatium and the inconspicuous tip of the *o. s.* $\times 2\frac{1}{2}$.

Fig. 15 *a.* The same. $\times 2$.

Fig. 16. *D. laxifolium*. Vegetative shoot. $\times 2$.

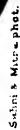
Fig. 17. The same. Transverse section of a stem showing six zones of wood in a radial thickness of 0.35 mm. Note the total absence of secretory sacs. $\times 32$.

Fig. 18. The same. Transverse-section of a leaf. The mesophyll is remarkably lacunar; there is no secretory canal; the transfusion tissue forms an arc over the vascular bundle. \times about 60.

Fig. 19. The same. Detached uni-ovulate strobilus. The seed with its hooked micropylar beak is seated erect on a receptacle formed of tumid bracts. The epimatium is just visible as a partial cup on the left. $\times 2$.

Fig. 20. The same. Longitudinal section of a young strobilus, showing the single terminal ovule with a columnar nucellus and hooked micropylar tube with an overhanging upper lip. Megaspore in free-nuclear stage. The margins of the epimatium overlap each other on the ventral side of the ovule, for they are cut in two distinct layers on that side. *No sign of an ovuliferous scale is visible on the surface.* Fertile bract, already much enlarged, showing papillate surface. $\times 27$.

Fig. 21. The same. Transverse section of a megastrobilus, through the basal part of an ovule. Much-enlarged epimatium (with wide air-space) enveloping the ovule. Megaspore in free-nuclear stage. The epidermis of one of the sterile bracts clearly shows its papillate character. $\times 32$.



On some New Species of Chlamydomonadaceae.¹

BY

F. E. FRITSCH

AND

FLORENCE RICH.

With three Figures in the Text.

THE material which forms the subject of the present communication was collected in December, 1921, by Miss M. Wilman from the surface of a vessel containing liquid manure and exposed to sunshine at the Museum, Kimberley. The colour is described as grass-green. The sample proved to be a practically pure growth of a number of different species of Chlamydomonadaceae. It formed one of a large collection from Griqualand West that is at present in course of investigation, and was preserved in the usual way in dilute formalin.

Material of this kind is frequently only of value if it happens to have been in a suitable state at the time of collection and was preserved without delay. This appears to have been the case in the present instance, as the sample in question not only contained a very large number of ciliated individuals, frequently showing with considerable clearness the structure of the protoplast, but also included many division-stages. It is probable, alone in view of the place of collection, that the preservative was added almost immediately, and we are no doubt justified in concluding that the individuals present show the normal structure and not a pathological condition such as often obtains, especially in hot regions, if the addition of the preservative is delayed. In view of the excellent state of the material and the fact that so little is known about the Chlamydomonadaceae of Africa, we have undertaken a detailed study of the three principal forms present.

¹ From the Botanical Department, East London College, University of London.

Polytoma caudata, n. sp. (Fig. 1).

This species, while the largest (usually 15–20 μ long and 6–10 μ broad) and most striking of the three to be described, was the least frequent. The general shape of the individuals is elongate-obovate, the anterior end being broadly rounded, the posterior end bluntly pointed with a rounded extremity (Fig. 1, A and B). The posterior end is not uncommonly somewhat set off, appearing as a short blunt tail, and it is to this feature that the specific name refers. Occasional specimens are more sharply pointed at the back end, and the latter is not uncommonly slightly curved to one side. Apart from this, the individuals are symmetrically developed about the longitudinal axis, i.e. they are circular in cross-section. The cell-membrane is everywhere of some thickness, but generally thicker at the front end than at the sides, and in the adult individual always strongly thickened at the posterior end, although the extent of this posterior thickening is very variable; in extreme cases it may make up one-third of the length of the entire cell. Young individuals possess a perfectly thin membrane (cf. Fig. 1, I), and it seems that the thickening of the posterior end may be progressive and some indication of age. Judging by its whitish appearance and reaction to stains, the greater part of the thick wall is mucilaginous in character. There is no beak ('Membranwarze') at the front end.

The usual shape of the protoplast is obovate, although sometimes oblong. The posterior end does not show the same prolongation as is customarily exhibited by the membrane. The anterior extremity of the protoplast is produced into a slight hyaline beak from which the two rather coarse cilia arise (Fig. 1, A, B, and F); their basal portions, traversing the thick membrane, either run parallel or diverge more or less sharply. The cilia are about as long as the body of the individual. Nearly all the numerous individuals examined possessed cilia.

As a general rule the whole of the peripheral cytoplasm is occupied by large oblong starch-grains; careful focusing, however, shows that a narrow marginal zone of the protoplast is always devoid of them. Occasional specimens harbour the starch-grains only in the posterior half (Fig. 1, B), while now and again an individual is found that completely lacks them. It is with some hesitation that we have referred the form under discussion to the genus *Polytoma*, but we have been unable to obtain any evidence of the presence of a chloroplast. The starch-grains are always confined to the peripheral region of the cytoplasm, a larger or smaller part of the central cytoplasm being altogether devoid of them. A study of specimens stained with Haidenhain's haematoxylin has failed to disclose any differentiation between the central and peripheral regions. The habitat is one likely to be suitable for saprophytes. Moreover, the large starch-grains are much more like those usually found in *Polytoma Uvella* than the small grains found in

the stroma of species of *Chlamydomonas*. Nevertheless, the assignation cannot be regarded as satisfactorily settled until living individuals of the species have been observed.

The nucleus is a moderately large rounded body occupying the central cytoplasm (Fig. 1, A and B), but commonly lying a little nearer the front than the back end. It often shows up very clearly in unstained material. Two small vacuoles can often be recognized at the anterior end, just

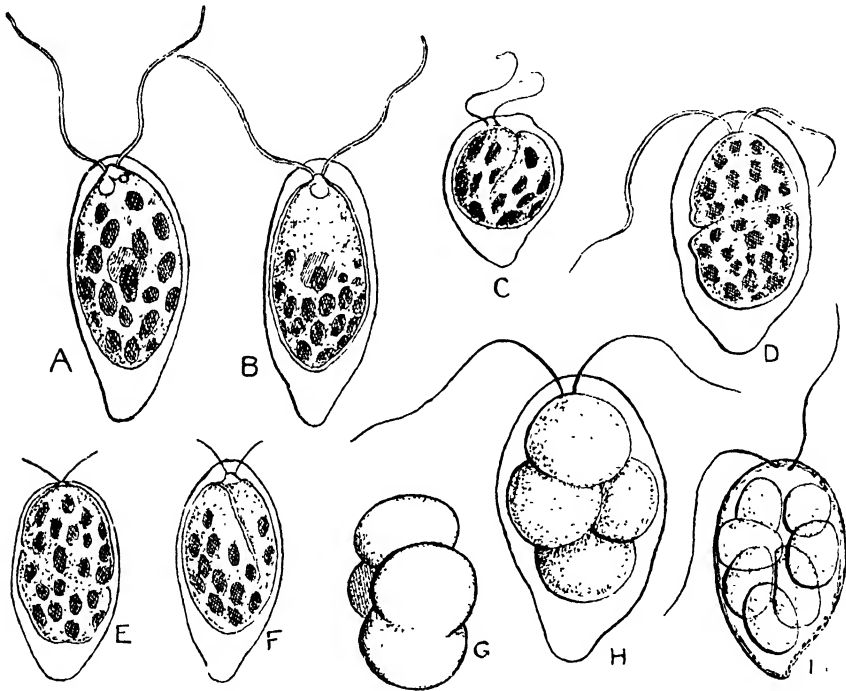


FIG. 1. *Polyoma caudata*, n. sp. A and B, two vegetative individuals. C-F, various stages in division. G-H, individual showing division into four; G, the contents of H seen from a slightly different angle. I, division into eight. (C, E, and F $\times 1,000$; I $\times 1,200$; the remainder $\times 1,500$).

beneath the protoplasmic beak (Fig. 1, A and B), and these are probably contractile vacuoles; they appear to lie in the same plane as the cilia. No other vacuoles were observed. An eye-spot seems to be lacking.

The smallest individuals noted had an oblong protoplast, whose length (9-12 μ) was about twice that of the breadth (5-6 μ). It would seem that for a time growth of the individual may take place mainly in the longitudinal direction, since many are to be found which are long and narrow. Finally increase in breadth seems to occur, and only such broad individuals (21-24.5 long, up to 15 μ broad) were found dividing. Division into two, four, eight, or sixteen daughter-cells has been observed, and in all cases the cilia are still plainly discernible on the parent individual. *P. caudata* thus

agrees with *P. Uvella* in dividing during movement. The furrow which initiates the first division may either be obliquely longitudinal (Fig. 1, C and F) or obliquely transverse (Fig. 1, D and E); when the former is the case, it passes to one side of the protoplasmic beak from which the two cilia of the parent arise (Fig. 1, F). The products usually still remain disposed obliquely when the division is complete (Fig. 1, E), although they were occasionally found placed symmetrically one in front of the other. It would seem that subsequent divisions are likewise often slightly oblique, although in successive perpendicular planes, but we have been unable to arrive at a perfectly clear conclusion with reference to this point. The new individuals were always oblong and provided with a close-fitting membrane. No other reproductive stages were observed.

Apart from the resemblances to which attention has been drawn above, the new species has not many points in common with *P. Uvella*, Ehrenb. There is a superficial resemblance to *Chlamydomonas caudata*, Wille, but this has the typical chloroplast of that genus and differs in other important respects.

2. *Chlamydomonas dorsiventralis*, n. sp. (Fig. 2).

The outstanding characteristic of this species is its dorsiventral construction. When viewed in the plane of the cilia (i.e. when the latter are seen stretched out to the right and left, Fig. 2, A, D, and E) the individual appears approximately symmetrical. The outline of the cell is then narrowly oblong, obovoid, or rarely almost subcircular, the lateral margins being subparallel or more or less convex and the posterior extremity rounded or bluntly pointed. The membrane is more or less appreciably thickened at this end of the cell, but elsewhere it is relatively thin. When the individuals are viewed in a plane at right angles to that containing the cilia, they present quite a different and characteristic appearance (Fig. 2, F). One of the lateral margins now appears flat and the other more or less markedly convex, whilst either extremity is bluntly rounded; sometimes the one margin is even slightly concave, so that the individual looks bean-shaped. In this profile view the cilia usually arise near the flat surface, but this is not always the case. It has not been possible to obtain a clear end-view of this species, but such approximations as have been seen would indicate that the cross-section is subcircular with a slight flattening on one side.

The dorsiventrality of the individual is also evident in the structure of its protoplast. The chloroplast occupies nearly the whole length of the cell and has the form of a strongly curved parietal plate, the somewhat irregular edges of which just fail to meet (Fig. 2, A and D); the slit between the edges often runs a little obliquely, and lies about in the middle of the flattened surface of the individual. It is readily seen when specimens are

viewed in the plane of the cilia with their flattened surface facing the observer (Fig. 2, A and D). On the other hand, when the convex surface is uppermost, the chloroplast appears entire (Fig. 2, B, C, and E). It is on this side of the cell that the single large pyrenoid is located, either midway between the two extremities of the cell or commonly a little nearer the posterior end (Fig. 2, D). The pyrenoid thus occupies a parietal position against the convex edge of the individual (cf. Fig. 2, F), but when the latter is viewed in the plane of the cilia the pyrenoid usually appears median

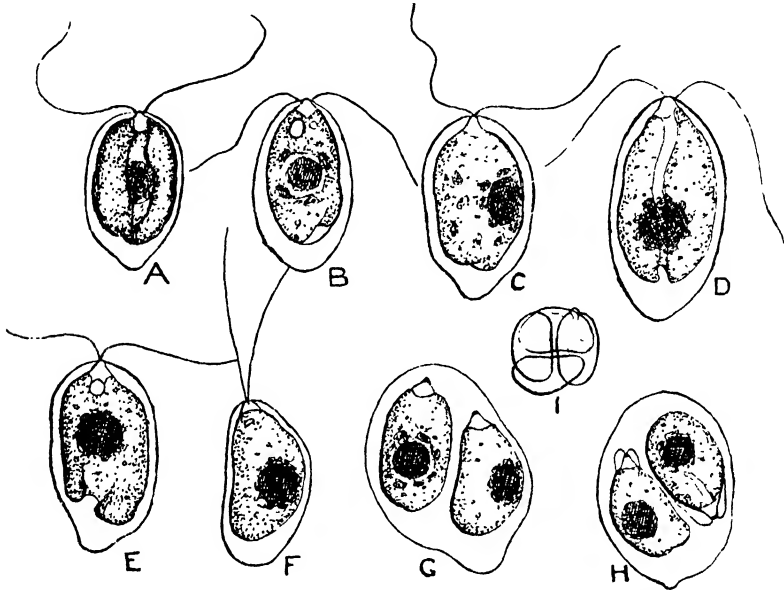


FIG. 2. *Chlamydomonas dorsiventralis*, n. sp. A-F, vegetative individuals seen from various points of view; A and D, median, from the flattened surface showing the slit between the two edges of the chloroplast and the median pyrenoid; B, C, and E, median, from the convex surface; F, side view. G and H, division into two; in H the cilia are visible on the daughter individuals. I, arrangement of the products after division into four. (All figures $\times 2,400$ about).

(Fig. 2, A, B, D, and E). The pyrenoid does not, however, invariably occupy just this median position; sometimes it is shifted a little towards one side or the other, with the result that it may not appear strictly parietal when the individual is seen in profile, or strictly median when seen in the other plane. A peculiarity noted in many, but not in all, of the individuals is the presence of a slight notch in the posterior portion of the chloroplast, often a little towards one side (Fig. 2, B, D, and E); when the notch is lacking, the posterior end of the protoplast is commonly somewhat oblique. The chloroplast occasionally contained a good deal of stroma-starch in the form of relatively large grains.

There is a prominent protoplasmic beak at the front end, from which the two cilia arise. This beak usually extends almost up to the outer limit

of the cell-membrane at this point, but there is no equivalent beak on the cell-wall itself. The cilia are generally a little shorter than the body of the cell and are relatively fine. The nucleus has not been clearly distinguished, but appears to consist of a small spherical body located near the pyrenoid, either behind or in front of it. Two small vacuoles (no doubt contractile) occur in the usual position just beneath the beak, lying in the plane of the cilia (Fig. 2, B). The dimensions of the cells are: length, 7–12 μ ; breadth, 5–6.5 μ .

Division, at least in this material, seems commonly to take place only into two, since in several such cases the presence of cilia on the daughter individuals was established (Fig. 2, H). The dividing individuals, as customary in *Chlamydomonas*, lose their cilia prior to division. No early stages of division were seen, and the two daughter protoplasts were always found lying side by side, with their long axes more or less parallel to that of the parent (Fig. 2, G and H). In many, but not in all, cases the two daughter individuals were so arranged that their respective protoplasmic beaks bearing the cilia were directed in opposite directions (Fig. 2, H), and not at all uncommonly one daughter individual lay with the pyrenoid median, while the other showed it parietal against the convex edge of the cell (Fig. 2, G and H). In fact such division-stages often afforded the best specimens for contrasting the appearance of the individual in its two positions. In the few cases in which division into four was observed, the long axis of two of the individuals was placed at right angles to that of the other two (Fig. 2, I).

A number of species of *Chlamydomonas* are known in which the chloroplast takes the form of a parietal plate, as, for instance, *C. Kuteinikovi*, Gorosch., *C. Dilli*, Dang., *C. mucicola*, Schmidle;¹ all of these are, however, as far as the available data go to show, radially symmetrical. G. S. West² has described a *C. elegans*, in which one side of the ovoid-ellipsoid cells is more convex than the other, but it is not clear from the figures and description whether the plate-shaped chloroplast shows any special location in the cell, as it does in *C. dorsiventralis*. As far as we are aware the latter species is at present unique in its marked dorsiventral construction.

3. *Chlamydomonas truncata*, n. sp. (Fig. 3).

This was the commonest form in the material; the majority of the individuals were, however, in a Palmella stage, and relatively few in the motile condition. The latter varied considerably in shape, but were usually more or less oblong, rounded at the posterior end, and characteristically truncate at the anterior (Fig. 3, A–C); the length is 10–14 μ and the breadth

¹ Goroschankin, Bull. Soc. Imp. Sci. Nat. Moscou, 1891, p. 117; Wille, *Nyt Mag. f. Naturvidensk.*, xli, 1903, p. 132; Schmidle, Ber. Deutsch. Bot. Ges., xxi, 1903, p. 349.

² *Journ. of Bot.*, 1915, p. 77.

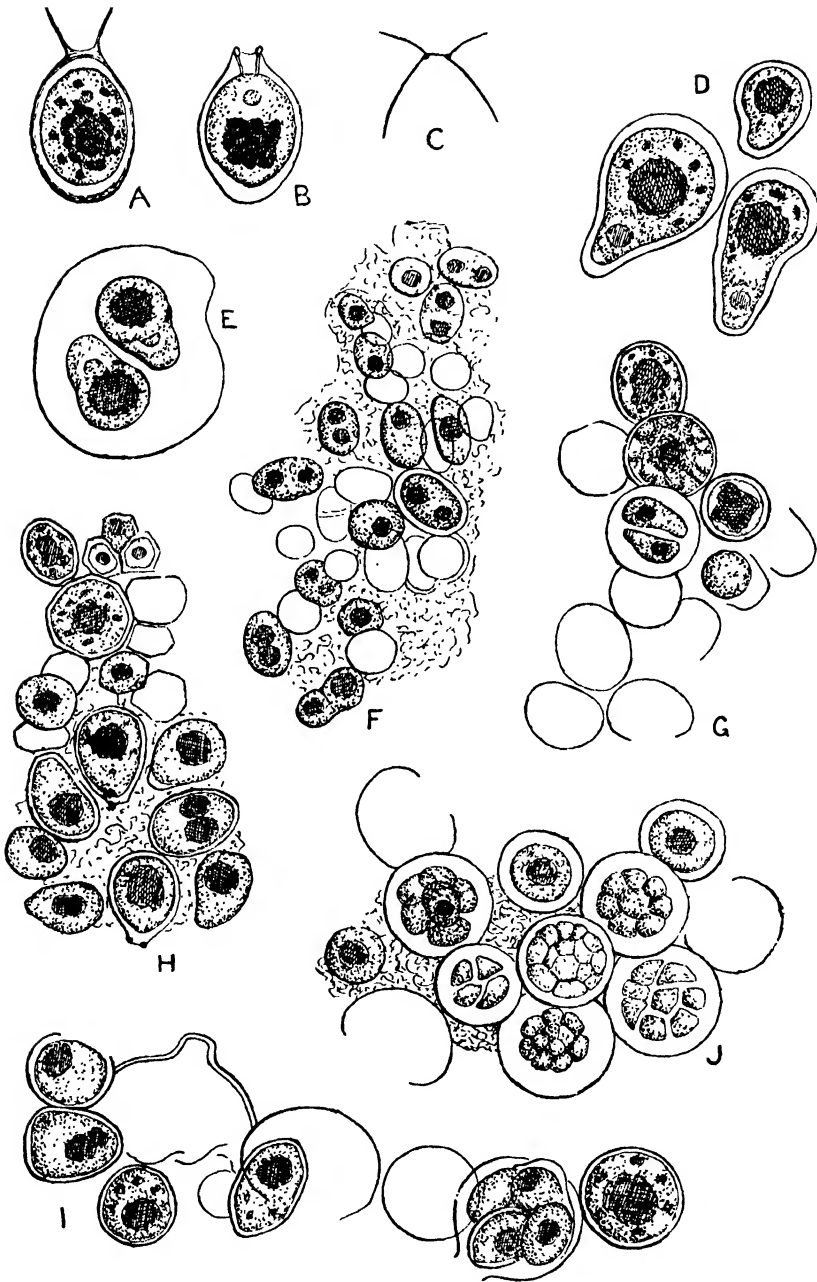


FIG. 3. *Chlamydomonas truncata*, n. sp. A and B, ordinary vegetative individuals. C, front end of another individual, showing the mode of insertion of the cilia. D, enlarged individuals with protruded front ends. E, division-stage of same. F-J, Palmella stages; in H showing form of normal individual, in F and J stages in division, in G and I empty cells from which the contents have escaped after division. (A and B $\times 1,500$; D and E $\times 1,200$; the remainder $\times 750$.)

6–9 μ . The protoplast shows the structure typical for the genus, viz. a basin-shaped chloroplast with a strongly thickened floor harbouring a large median angular pyrenoid, usually densely surrounded by starch, while the small nucleus is situated in the anterior part of the protoplast in front of the pyrenoid.

There is no protoplasmic beak, the two cilia arising near together, but not in immediate contiguity, from the front end of the protoplast (Fig. 3, B); they diverge slightly and emerge through the cell-wall at the outer edges of the truncate front margin of the cell. Here one can usually recognize two minute thickenings of the wall, one at the point of emergence of each cilium. While the basal portions of the cilia are often preserved, we have altogether failed to obtain a clear view of the distal portions; it would seem as though these distal parts were very delicate in contrast to the bases. The front end of the cell often appears to be somewhat mucilaginous, as there is nearly always a quantity of adhering foreign matter, and this has also rendered a study of the cilia very difficult. The cell-wall is generally somewhat thickened fore and aft, but not appreciably so at the sides of the individual.

Division in this species, or at least in this material, appears only to occur in the *Palmella* stage, but here it is abundant. The stages in question comprise large numbers of usually rounded cells of uniform (Fig. 3, F) or very dissimilar size (Fig. 3, H) embedded in a thin and usually quite structureless mucilage; the diameters of the cells vary between 6 and 15 μ . These stages are readily recognized as belonging to this species because they show the same cell-structure with a large angular pyrenoid, and because occasional cells show the truncate anterior extremity with the membrane thickenings marking the points at which the cilia usually emerge (Fig. 3, H). Multiplication of the cells within these *Palmella* stages takes place by division into two, four, eight, or more numerous portions (Fig. 3, F, J). Sooner or later the parent membrane bursts open on one side (Fig. 3, G, J) and the products are liberated, either as motionless units or as motile individuals. Cases were found in which such *Palmella* stages included large numbers of empty cells whose contents had vanished (Fig. 3, G), and others again in which the small division products were found lying outside the empty parent membrane. Remains of such membranes are commonly to be found within the mucilage of the *Palmella* stages (cf. Fig. 3, I) and may well in great part give origin to it. When the products of division are not immediately liberated as motile individuals, they evidently in part undergo great subsequent increase in size, with the result that they may become angular by mutual pressure. Division may take place again and again, and thus extensive masses arise.

Occasional cells of the *Palmella* stages may attain very large dimensions (18–21 μ long, 14–16 μ broad), and such frequently exhibit a much-

protruded anterior extremity (Fig. 3, D), in which the nucleus is often very distinctly seen. In these distorted cells retraction of the protoplasm towards the posterior region gradually takes place, and this may be followed by division with the production of normal individuals (Fig. 3, E). Commonly, however, the contracted protoplast becomes enveloped by a new membrane, and such membrane formation may sometimes take place several times in succession, since one may occasionally find in the *Palmella* stages empty cells with several irregular membranes from which the contents have vanished. Large cells, with similar protruded anterior ends, were sometimes found apart from the *Palmella* stages, and it seems that they may arise directly from the normal individuals.

This species shows considerable resemblance to *C. Ehrenbergii*, Gorosch.,¹ but it is sharply distinguished by its truncate front end, the absence of a protoplasmic beak bearing the cilia, and the evident readiness with which *Palmella* stages are produced. It may also be compared with *C. urccolata*, Printz,² which has a somewhat similar form.

It is of interest that, of three species apparently existing under identical conditions, only one should show an extensive development of the palmelloid phase. This is open to two interpretations, viz. either that this species forms a *Palmella* stage as a normal feature of its life-cycle, or that the conditions that call forth a *Palmella* stage in this case are not identical with those which induce it in the two other forms. It may well be that *C. truncata* is one of those species (like *C. Kleinii*, Schmidle) in which the *Palmella* stage is the normal vegetative condition, motile individuals only being formed at rare intervals.

It is noteworthy that all three species show more or less marked thickening of the membrane at certain points, a feature which may be related to the habitat conditions. Were it not for the material being otherwise apparently normal, one might be inclined to look upon this thickening as evidence of abnormal conditions.

SUMMARY.

Three new species of *Chlamydomonadaceae*, obtained from a vessel containing liquid manure at the Museum, Kimberley, are described, viz. *Polytoma caudata*, n. sp., *Chlamydomonas dorsiventralis*, n. sp., and *C. truncata*, n. sp. The first of these is essentially distinguished by its shape; the second is characterized by a marked dorsiventral construction, involving not only the shape of the cell but also the arrangement of its parietal plate-shaped chloroplast; while the third is peculiar in the truncation of the anterior end and an apparently dominant *Palmella* stage.

¹ Goroschankin, loc. cit., p. 128.

² Printz, Videnskapselskap. Skrift., i, Mat. Nat. Kl., 1913, No. 6, p. 19.

The Coleoptile Bundles of Indo-Abyssinian Emmer Wheat (*Triticum dicoccum*, Schübl.).

BY

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With five Figures in the Text.

WHILE investigating the leaf anatomy of the embryos and young plants of some 2,000 wheats representing all species and races, I observed that a small number of them possessed from two to six nerves or vascular bundles in the coleoptile, instead of two, which is the characteristic number in wheats and in the Gramineae generally (Figs. 1-5). The wheats exhibiting this peculiar anatomical character were found to be wild Emmer (*T. dicoccoides*), cultivated Indian and Abyssinian varieties of Emmer (*T. dicoccum*) with a fragile rachis, a few wheats with a tough rachis from the highlands of Abyssinia, and a form known as Black Persian, obtained from Messrs. Haage and Schmidt, of Erfurt, Germany.

From an extended study of these wheats I concluded that they were all closely related, and that the cultivated forms were best classified as varieties of the Emmer race (*T. dicoccum*, Schübl.), dividing them into a *Speltae* section with brittle rachis, and a *Tenaces* section with a tough rachis (1).

I have recently received a further collection of Abyssinian wheats, also three varieties from Professor Zhukovsky, of the Polytechnic Institute, Tiflis, which he found in cultivation in the mountainous regions of Transcaucasia; all these have the characters of the *Tenaces* section of the Emmer wheats, and possess more than two coleoptile bundles.

In the following lists are given the number of coleoptile bundles in about twenty grains from pedigree lines of each of the varieties named.

During the last four years (1922-5) I have carried on investigations to determine how far this peculiar anatomical character is hereditary in Black Persian wheat (*T. dicoccum*, var. *persicum*, Haage and Schmidt's form), and

have endeavoured to obtain plants breeding true in respect of the number 2, 3, or 4 coleoptile bundles. (In making the determinations of the number of bundles, the grains are germinated on filter-paper in Petri dishes, and when the plumules reach a length of 5–10 mm. transverse hand-sections are cut and the number of bundles counted under a low magnification. The embryos do not suffer by this treatment, the grains when sown yielding healthy plants.)

Variety.	Number of Grains tested.	No. of Grains with				
		2	3	4	5	6 bundles.
<i>Ajar</i>	21	—	—	17	4	—
"	20	13	5	2	—	—
"	22	1	3	17	1	—
<i>nigro-Ajar</i>	20	—	—	20	—	—
<i>Browni</i>	20	10	7	3	—	—
<i>uncinatum</i>	17	—	4	10	3	—
"	20	2	4	8	6	—
"	20	6	7	7	—	—
<i>Arraseita</i>	17	6	6	5	—	—
"	20	20	—	—	—	—
<i>pseudo-Browni</i>	17	2	4	6	3	2
<i>pseudo-uncinatum</i>	16	2	1	10	3	—
<i>tomentosum</i>	22	1	5	11	4	1
<i>amharicum</i>	17	9	6	2	—	—
<i>pseudo-tomentosum</i>	21	1	7	9	3	1
<i>Grabhami</i>	19	7	6	3	1	2
<i>rubescens</i>	20	5	3	9	2	1
"	23	6	6	5	4	2
<i>rufescens</i>	21	2	5	9	5	—
"	22	6	8	8	—	—
<i>pseudo-rubescens</i>	20	6	8	6	—	—
<i>pseudo-rufescens</i>	22	8	9	5	—	—
"	20	2	5	10	3	—
<i>vulpinum</i>	20	5	14	1	—	—
"	20	6	6	4	3	1
<i>rubrivillosum</i>	16	5	7	4	—	—
<i>ethiopicum</i>	18	3	2	12	1	—
<i>persicum</i>	27	17	8	2	—	—
<i>Zhukovsky's stramineum</i>	31	10	9	12	—	—
" <i>rubiginosum</i>	23	22	1	—	—	—

A single ear of a plant was chosen, its grains germinated, and the number of coleoptile bundles in each embryo determined. All the grains with embryos possessing two bundles were then sown in one row, and the others with three and four bundles were also sown in separate rows. After harvest single ears of several plants from each row were examined, and the grains with embryos having two, three, or four bundles respectively were sown in separate rows as before, the process being repeated a third season.

It was found that plants raised from embryos with two coleoptile bundles produced grains some of which possessed two bundles, others three or four, and plants from embryos with three or four bundles gave similar results. No plant was discovered in which all its grains had embryos with the same number of coleoptile bundles; every plant, whatever its origin, exhibited the same polymorphism in respect of bundle number.

Occasionally an ear was found in which the embryos of all the grains had two coleoptile bundles, but the rest of the ears on the same plant yielded grains having two, three, or four bundles.

Plants raised from embryos with two coleoptile bundles were more vigorous and showed a higher tillering power than those from embryos with three or four bundles, the average number of grains per ear being also greater in plants from embryos of the former class. In any plant the number of the grains with embryos possessing two, three, or four bundles, respectively, varies within wide limits and there appears to be little or no correlation between the coleoptile bundle number of the parent and its offspring.

Examples of the progeny of a plant (E₂) grown from an embryo having two coleoptile bundles, and that of a plant (E₄) from an embryo with four bundles, are given below.

No. of Ear.	Plant (E ₂) with 2 Bundles. Coleoptile Bundles.			Plant (E ₄) with 4 Bundles. Coleoptile Bundles.		
	2	3	4	2	3	4
	No. of Grains.			No. of Grains.		
I	30	9	1	26	8	—
II	32	1	1	24	3	—
III	42	9	1	29	7	—
IV	33	7	1	18	2	—
V	29	12	—	25	2	—
VI	28	5	1	23	—	—
VII	17	1	—	24	—	—
	25	4	—	27	—	1
	20	8	1	13	3	1
	256	86	6	209	19	2
	80.5	17.6	1.9	90.9	8.2	0.9
	4.13 : 1			9.95 : 1		

More than 5,000 embryos of grains from 214 ears of a pedigree line of this variety were examined, namely, grains from

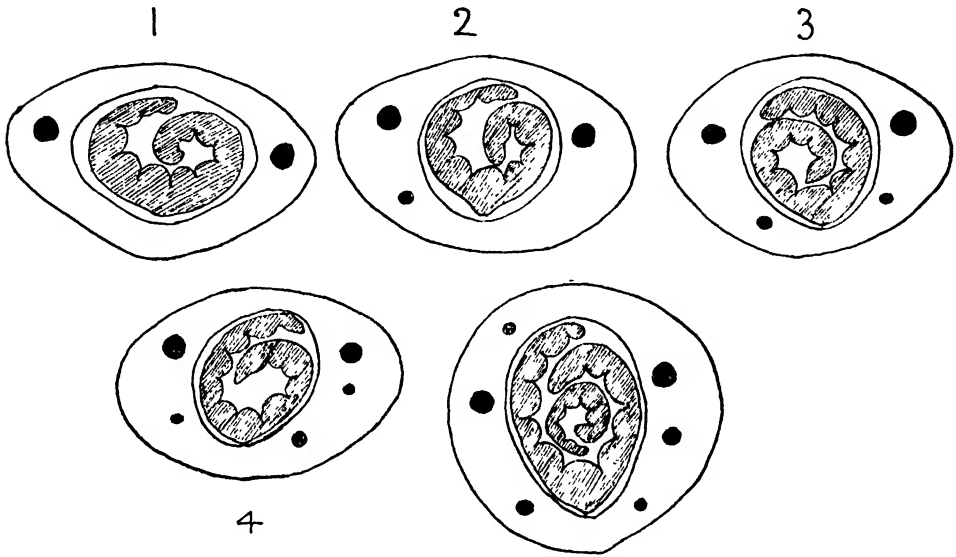
- (1) single ears of 91 plants each grown from an embryo with 2 bundles,
- (2) single ears of 82 plants from embryos with 3 bundles,
- (3) single ears of 41 plants from embryos with 4 bundles.

The results are presented in the following table :

No. of Bundles in Parent.	No. of Plants. A single Ear of each examined.	Total No. of Embryos.	Coleoptile Bundles.		
			2 No. of Embryos.	3 No. of Embryos.	4 No. of Embryos.
2	91	2287	1783	428	76
3	82	2095	1648	377	70
4	41	953	720	200	33
2	Ratio			3.54 : 1	
3	Ratio			3.68 : 1	
4	Ratio			3.02 : 1	

The ratio of the number of embryos with two coleoptile bundles to the number with more than two is about 3.5 : 1, and suggests that the original plant was heterozygous, the character of two bundles being dominant ; more than two, recessive. The ratios, however, are not convincing, and it has been found impossible to isolate either pure recessives with more than two bundles, or pure dominants with only two bundles.

In discussions upon the homology of the parts of the grass embryo the



FIGS. 1-5. 1-3. Abyssinian Emmer Wheat (*Triticum dicoccum*, var. *persicum*).
4 and 5. *Triticum dicoccum*, var. *uncinatum*.

almost universal occurrence of only two bundles in the coleoptile has been used as an argument in support of the view that the coleoptile is a ligular structure. In dismissing the conclusions of Bruns and Coulter that the coleoptile is a leaf, Worsdell (2) says, 'The possession by the coleoptile of two widely separated vascular strands which are situated much nearer to the two margins than they are to each other strongly suggests a ligular structure formed by the union of stipules. If this organ represented an independent (first plumular) leaf this type of venation would certainly not occur, but, instead, there would be two or three veins placed at equal distances from each other and from the margins.' The presence of several bundles in the coleoptiles of these wheats provides the evidence which was lacking when Worsdell wrote, and upholds the contention of Bruns that the coleoptile is a leaf which may be homologous with the prophylls of the plant, and of which it may be considered the first.

My thanks are tendered to Mrs. L. Proctor, B.Sc. (*née* Grieve), and to Mr. A. S. Thomas, B.Sc., for their invaluable assistance in the laborious task of examining the very large number of embryos mentioned in this communication.

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2. WORSDELL, W. C. : The Morphology of the Monocotyledonous Embryos and that of the Grass in particular. Ann. Bot., 1916, vol. xxx, p. 510.

Fertilization and Embryogeny in *Theobroma Cacao*, L.

BY

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With twenty Figures in the Text.

EXAMINATION of the literature reveals a dearth of information about the biology of *Theobroma Cacao*, L., which is surprising when one considers its importance as a tropical crop. Even the method of pollination is not fully understood, though a substantial advance in our knowledge on this point has recently been made by Harland (1). Hunger (3) suggested, after reviewing the evidence, that some form of parthenogenesis might possibly occur, since nobody had ever seen pollen tubes in sections of the style. Kuyper (4) worked out the development of the ovule and embryo-sac, which he showed to be normal, but was unable to finish his studies, and did not observe fertilization.

Genetic studies by Harland (2) have proved that normal Mendelian segregation of certain characters occurs in the seedling progeny of some cacao trees, whilst the progeny of other trees comes true to the parent type. In either case seedlings from some of the fruits of a tree may show segregation in such a manner that vicinism is clearly indicated. Two facts are thus established: first, that fertilization does occur, and second, that both self- and cross-fertilization are possible. A description of the process of fertilization, however, is still lacking, and such it is the aim of the present paper to provide.

Methods.

Flowers were taken from twelve-year old trees growing at St. Augustine Experiment Station; several trees were used, and no differences were noticed in the material from them. When material was to be fixed, all open flowers were removed from the tree just before nightfall, so that all those open in the morning should be fresh. Pollinations were made by hand, usually

between eight and nine o'clock in the morning, and at the required intervals afterwards the ovaries were fixed in the field, the perianth and staminal column being dissected away first. In stages where the ovary had begun to swell, it was pared on two sides with a razor to facilitate entry of the fixative, and for the later stages young seeds were dissected out of the pod, and fixed alone.

Considerable difficulty was experienced in finding a suitable fixative for the mature embryo-sac. A wide range of chromo-acetic mixtures, with and without osmic acid, all caused collapse and distortion. Mixtures of absolute alcohol and acetic acid were better, but not altogether satisfactory. Bouin's formula was finally selected as the best available. The difficulty was probably due to the large amounts of mucilage present in the tissues.

Material was dehydrated and embedded (in paraffin of m.p. 58° C.) through isobutyl alcohol, and cut nearly always at 5 μ . Heidenhain's haematoxylin was used for most of the staining, though safranin was also found useful.

Development of the Embryo-sac.

A number of young stages were examined, and found to correspond with the description and figures given by Kuyper (4). The course of events may be summarized briefly as follows:

The archesporial cell, probably originating in the hypodermal layer of the nucellus, becomes 'buried' by subsequent divisions, so that at meiosis it is covered by five or six cell-layers (Fig. 1). Four daughter cells are formed in a row, and the lowest functions as the megaspore; the other three degenerate, but are visible for a time as a darkly staining cap over the embryo-sac (Fig. 2). Divisions proceed regularly, the third giving rise to the usual complement of eight nuclei. The antipodal nuclei usually begin to degenerate at once, but remain recognizable for some time. The polar nuclei come together, but do not fuse until after fertilization.

When the embryo-sac is mature, the two synergidae are the most conspicuous of its contents. They lie side by side in the plane transverse to the ovary, and each has a large vacuole at its antipodal end. The egg-cell is larger, but poorer in cytoplasm.

There are also prominent in the sac a number of spherical bodies, which if the term is used in its wide sense are starch grains, as with iodine or chlor-iodide of zinc they stain reddish purple. In Canada balsam these grains become inconspicuous owing to similarity of refractive index, but their 'hilum' shows up as a dark spot if the cone of light by which they are illuminated is reduced. They do not stain with safranin, and only very faintly with iron-haematoxylin, until the third or fourth day after fertilization, when they stain brown with the haematoxylin; at the same time they become irregular in outline, and a little later they disappear altogether.

Grains much smaller, but giving exactly the same staining reactions, occur in the cells of the nucellus at its micropylar end, and in the epidermis of the

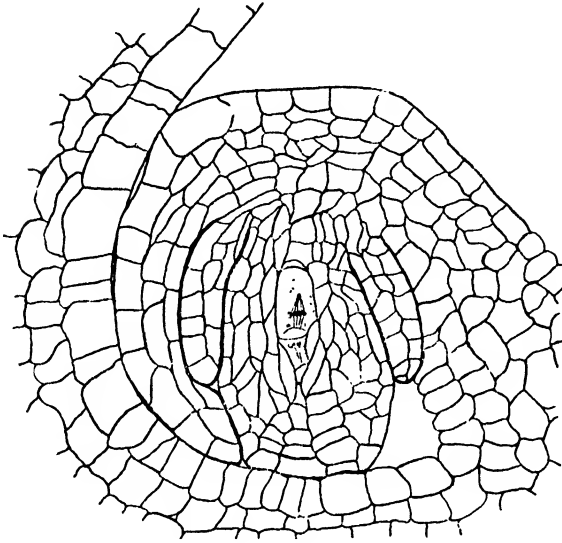


FIG. 1. Ovule showing meiosis of the megaspore mother-cell. $\times 450$.

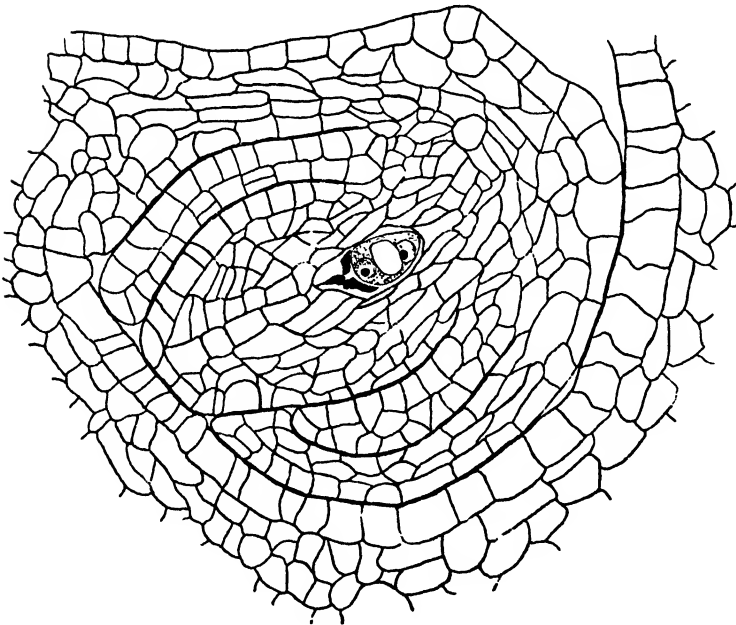


FIG. 2. Binucleate stage of embryo-sac showing degenerated megaspores. $\times 450$.

placentae, but nowhere else in the ovary. It may be remarked in passing that starch grains in the stem of the plant give the more usual blue colour with iodine.

The mature embryo-sac as seen in two adjacent sections is shown in Fig. 3. It measures about 70μ in length by 40μ in diameter at its widest part.

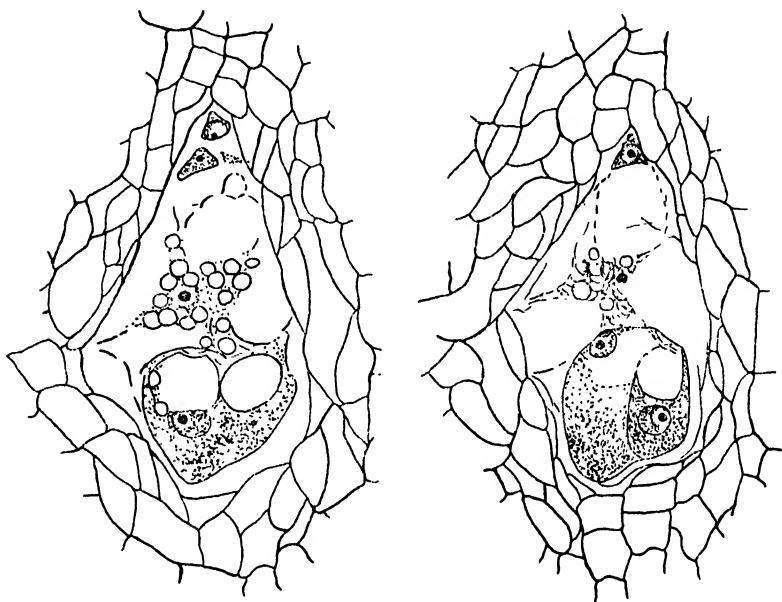


FIG. 3. Mature embryo-sac, as seen in two adjacent sections. $\times 725$.

Abnormalities.

Although the total number of ovaries sectioned was well over two hundred, very few indications of abnormality were found. In one case an embryo-sac from an unopened flower-bud contained sixteen nuclei, six at the micropylar end which seemed to be derived from the synergidae, five at the antipodal end, some of which were degenerating, and five in the middle part of the sac. Cases were described by Kuyper in which two archesporial cells had begun to develop, and at the side of the normal sac were the degenerating remains of a second. The present case did not appear to have originated in a similar way, however. Seeds giving rise to more than one plant occur occasionally in cacao, but no case of two perfect sacs in one ovule was observed in the preparations.

Chromosome Number.

The chromosome number of *Theobroma Cacao* was found by Kuyper to be eight in the reduction divisions of the megaspore mother-cell, and sixteen in dividing cells of root tips. Advantage was taken of some good figures which occurred in dividing cells of the nucellus to confirm this statement, and sixteen chromosomes were counted in several cases (Fig. 4).

Growth of the Pollen Tube.

Cacao pollen germinates readily in a saturated atmosphere, but usually will not do so in liquid water or solutions. If a number of fresh flowers are corked up in a glass tube for an hour or so, the pollen germinates in the anthers; or short pollen tubes may easily be obtained by dusting the grains

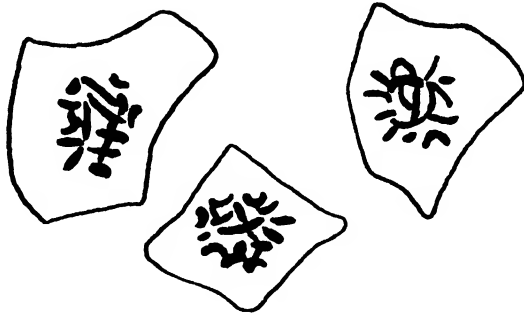


FIG. 4. Chromosomes at metaphase of division in cells of the nucellus. $\times 2,500$.

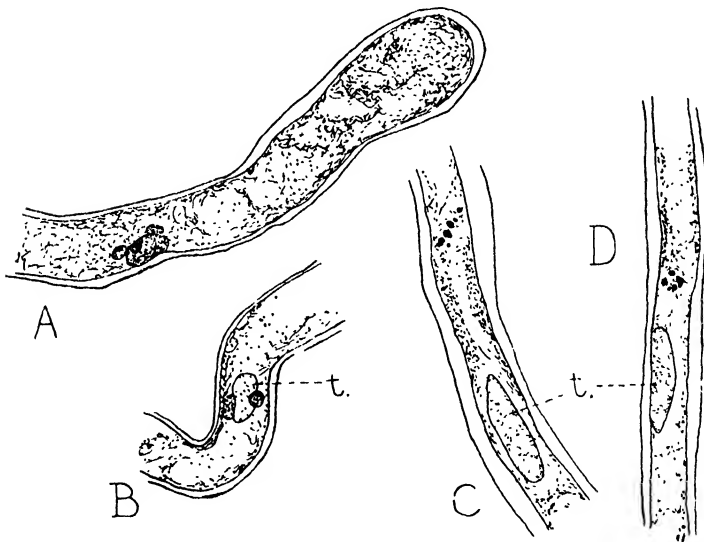


FIG. 5. Nuclei of pollen-tubes grown on agar, showing the male gametes at A and B, and division of the generative nucleus at C and D. *t.*, the tube nucleus. $\times 1,250$.

on a cover-slip and inverting over a Van Tieghem cell with a drop of water in the bottom. Better growth, however, is obtained on agar (1.5 per cent. to 2 per cent.) with 5 per cent. of cane sugar. Tubes were grown on thin films of this medium on slides, fixed in Bouin's fluid, and stained in iron-haematoxylin. Young stages did not give good results by this treatment, but in older ones it was quite possible to distinguish the nuclei within (Fig. 5).

The tube nucleus stains scarcely darker than the cytoplasm, but its outline is sufficiently distinct. It lies nearly always quite close to the gametes,

usually a little in advance of the latter, but in a few cases observed slightly behind them. In Fig. 5 tubes A and B were drawn from a preparation of pollen which had been allowed to grow for about six hours, whilst C and D were fixed after four hours. The difference in size of the tube nucleus is striking, but in each case the nuclei drawn were typical of the preparation as a whole.

In practically all the pollen-tubes of the second preparation the male nuclei are represented only by a number of black granules, as shown. There is little doubt that these are chromosomes, and therefore that the generative nucleus divides in the tube, although it was not actually seen in an undivided and resting condition. Whilst the course of events is not necessarily the same in the style as on agar, it seems probable that division of the generative nucleus takes place only a short time before fertilization.

When pollen is placed on the stigmatic lobes it germinates very quickly, and one hour later the tubes are nearly half-way down the style. The first of them begin to reach the ovules in about four hours, and they can be traced to the micropyles. Through the greater part of their course they travel over the surface of the epidermis lining the stylar canal and placentae, and not within the tissues. Nevertheless they have a disruptive effect on the style, the cells of which readily fall apart under gentle pressure and stain with ruthenium red in a manner suggesting hydrolysis of the middle lamellae by a cytase.

The structure of the ovary and the course of the pollen-tubes within it are shown diagrammatically in Fig. 6. It will be noted that there is a well-marked stylar canal, which opens into a cavity extending to the middle of the ovary, so that the placentation of the ovules is 'parietal' above and

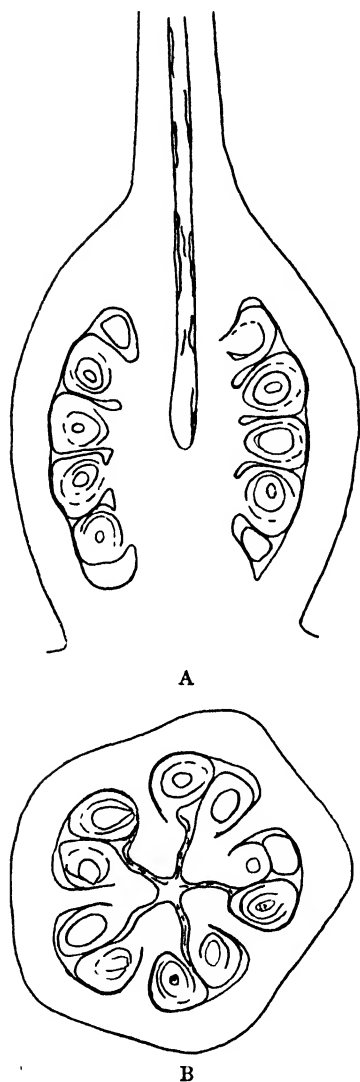


FIG. 6. A, median longitudinal section of ovary; B, transverse section of upper part of ovary. Both somewhat diagrammatic to show the course of the pollen-tubes. $\times 42$.

'axile' in the lower part of the ovary. Fig. 7 shows the condition of the tissues bordering the stylar canal before pollination and two hours after.

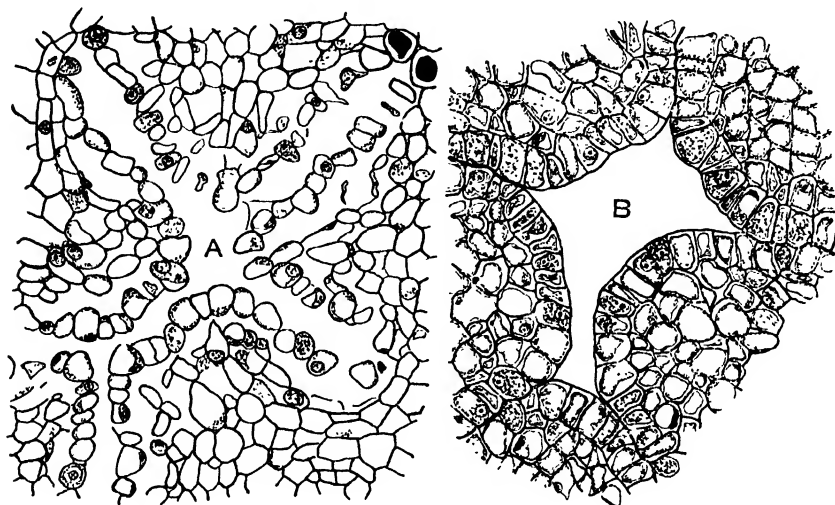


FIG. 7. Tissue bordering the stylar canal; A, in a pollinated style two hours after pollination; B, in an unpollinated style. $\times 525$.

Fertilisation.

The pollen-tubes are very slender (about 1 to 1.5μ in diameter when within the ovary), and on this account the actual point of entry was rarely seen. In cases where it was clear, as in that drawn in Fig. 9, it always occurred at the tip of the nucellus. In preparations of ovaries fixed five or six hours after pollination there are in most of the ovules densely staining bodies in the micropylar end of the embryo-sac which almost certainly represent some of the contents of the pollen-tube (Fig. 8). At the same time the micropylar ends of the synergidae often show an indistinct outline, indicating an alteration in their constitution caused by the proximity of the pollen-tube.

The male gametes have never been found in embryo-sacs at this stage, though large numbers were examined. It is, however, scarcely remarkable that such small bodies should be indistinguishable from the highly granular cytoplasm of the synergidae.

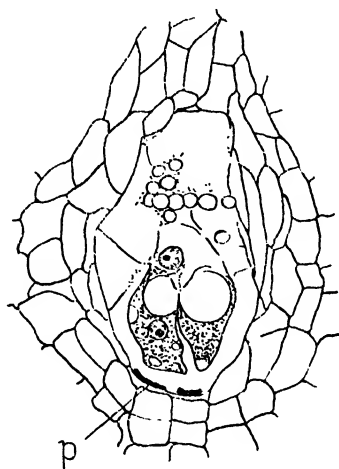


FIG. 8. Egg-apparatus six hours after pollination. *p.*, some contents of the pollen-tube. $\times 725$.

Two hours later, one of the synergidae is represented only by a shapeless mass, staining a deep black, whilst the other is still fairly normal (Fig. 9). Occasionally both have degenerated. Still the male nuclei cannot always be distinguished with certainty, but in some cases, as shown in Fig. 10, they are quite clear, one lying against the egg, and the other against the polar nuclei.

Thirty hours after pollination the egg nucleus has usually either two conspicuous nucleoli or indications of a male gamete by its side, whilst the endosperm nucleus is clearly a triple structure (Fig. 11). Fusion in the

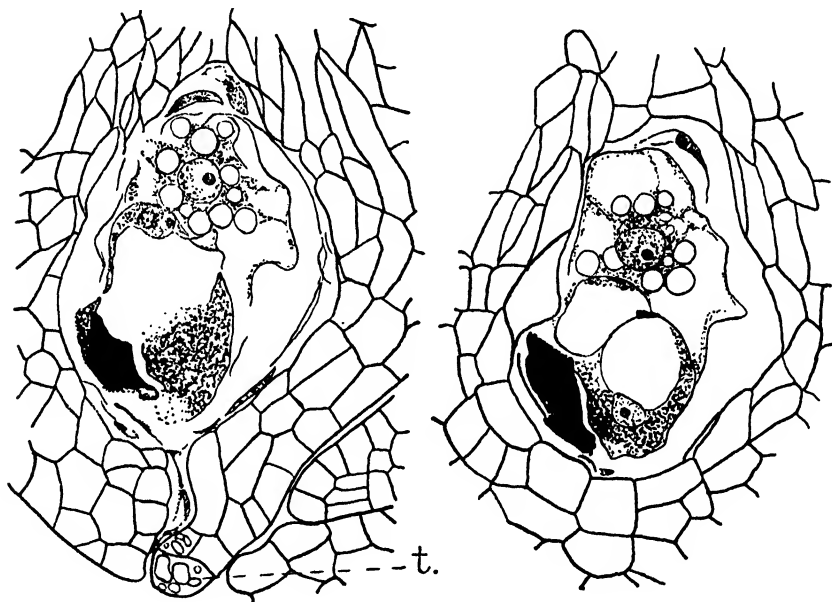


FIG. 9. Seven hours after pollination. Two adjacent sections showing the entry of the pollen tube and destruction of one synergida. *t.*, cells containing tannin. $\times 800$.

latter nucleus is in many cases still incomplete, so that one finds two nuclei in one section and the third in an adjacent section. The second synergida is now very poor in cytoplasm, though its nucleus still looks normal. The embryo-sac has enlarged somewhat, measuring now about 100μ by 60μ , and, since its contents have not increased at all, it looks almost empty.

The process of fertilization may, it is believed, be taken as normal. Unfortunately, the cases in which the actual union of the gametes was observed were very few, but that is probably due entirely to technical difficulties introduced by the small size of the male nuclei. The constancy with which, in ovaries fixed more than six hours after pollination, one synergida in each sac shows signs of degeneration, whilst in unpollinated ovaries of the same age or older this does not occur, leads to the conclusion

that the majority of the embryo-sacs receive pollen-tubes. That these tubes function alike may be inferred from the fact that in subsequent stages all the ovules in a given ovary are commonly in a similar condition.

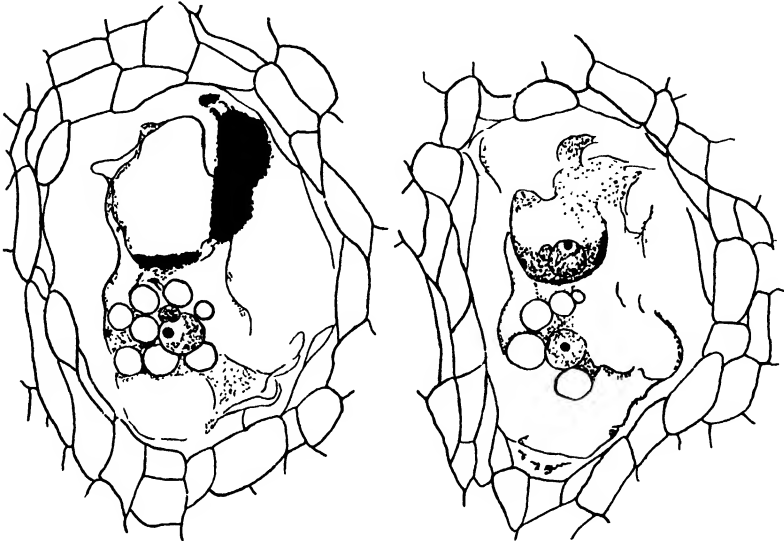


FIG. 10. Seven hours after pollination. One male nucleus against the egg, and the other near to the polar nuclei. $\times 800$.

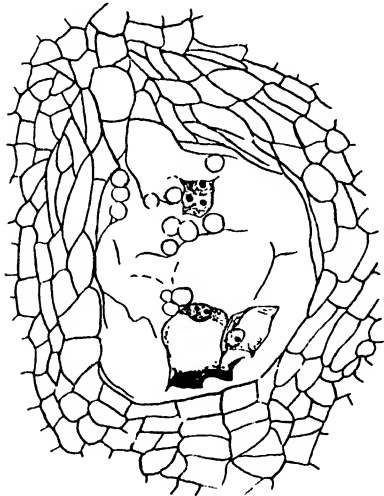


FIG. 11. Thirty hours after pollination. Composite drawing from three consecutive sections, showing fertilized egg, triple fusion nucleus, and the surviving synergida. $\times 480$.

The Endosperm.

The first division in the embryo-sac takes place sometimes on the third but more often on the fourth day after fertilization, and in preparations made then two endosperm nuclei are found. Fig. 12 shows a fortunate section,

fixed 120 hours after pollination, in which the second division is in progress. The mitotic figures were poorly fixed, but a count of the chromosomes was attempted, and gave a result of twenty-two, so that there can be little doubt that the nuclei are triploid.

A long and leisurely free-nuclear stage ensues, the first cell walls

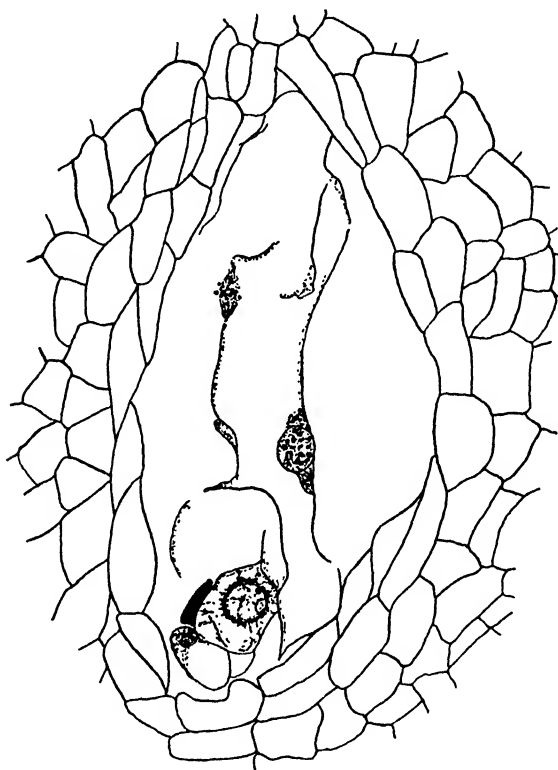


FIG. 12. 120 hours after pollination. The second division of the endosperm nucleus in progress. Egg apparatus drawn from other sections of the same embryo-sac. $\times 725$.

appearing at the micropylar end of the sac when the fruit is not less than fifty days old. Fig. 13 was drawn from a fruit about eighty days old, and shows that in the early stages there is a considerable development of the nucellus, forming perisperm, whilst up to the time of wall-formation the endosperm is a very thin layer lining the embryo-sac. From this point onwards the endosperm increases more rapidly, and the perisperm is soon reduced in proportion. During the free-nuclear period the divisions are not strictly simultaneous, but neighbouring nuclei are always in similar phases, and there is a gradation of phases from one end of the sac to the other.

The endosperm nuclei differ among themselves at all stages in the number of nucleoli which they possess, some having one, some two, and some three, as shown in Fig. 14. The presence of three distinct spiremes in

the first division of the primary endosperm nucleus has been reported in *Lilium* by Weniger (9), and in *Triticum* by Sax (8), whilst Nothnagel (6) found one nucleus in the third division of the endosperm in *Trillium grandiflorum* with three distinct chromatic groups, consisting of six chromosomes each. It was at first thought that the variation in the number of nucleoli in *Theobroma Cacao* might be consequent upon a similar loose union, persisting longer, or might even indicate the possibility in some cases of disjunction of the constituents. It was then noticed, however, that the nuclei of the nucellus and integuments have also frequently more than one nucleolus, though in their case the number rarely exceeds two. The condition is, therefore, probably characteristic of the plant, and its significance, if any, outside the scope of the present work.

A large number of mitotic figures were observed in the endosperm at the stage of Fig. 13; the great majority of them were quite normal.

The Embryo.

The first division of the fertilized egg was not observed, but apparently it occurs from forty to fifty days after fertilization. The ovules in young fruits from 1 to 5 cm. long are particularly difficult to fix, so that this stage could not be studied in detail.

In fruits 1 cm. long, or about eighteen days old, the undivided zygote can be found quite easily, and appears as shown in Fig. 15. Nineteen embryo-sacs in two different fruits were examined at this stage, and all were similar, though in many the egg-cell was even less conspicuous than in the example drawn. Fruits 1.5 cm. long gave a like result, and although preparations from fruits larger than that were mostly too poor to give definite evidence, a similar undivided cell was traced with certainty in

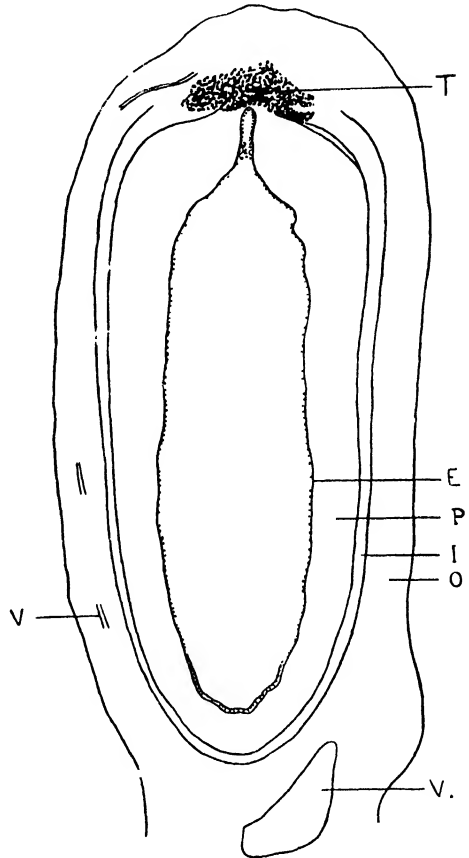


FIG. 13. Young seed, at stage of first wall-formation in the embryo-sac, from a fruit about eighty days old. E, embryo-sac; P, perisperm; I, inner integument; O, outer integument; T, chalazal cells filled with tannin; V, vascular bundles. $\times 14$.

a fruit 4 cm. long (about forty-three days old), whilst some ovules of a pod 4.6 cm. long appeared to have a binucleate cell in the same position.

The youngest embryos clearly recognizable as such were found in a pod 5.1 cm. long, or about fifty days old. Fig. 16 shows one of them, and comparison of this drawing with Fig. 15 leaves no room for doubt that the

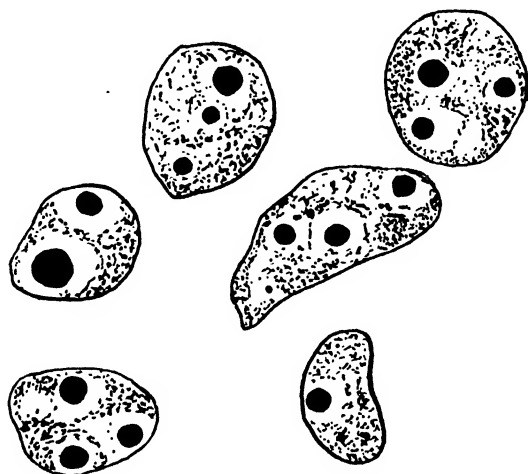


FIG. 14. Typical nuclei of the early stages of the endosperm, from a sac containing about thirty nuclei in all. $\times 1,250$.

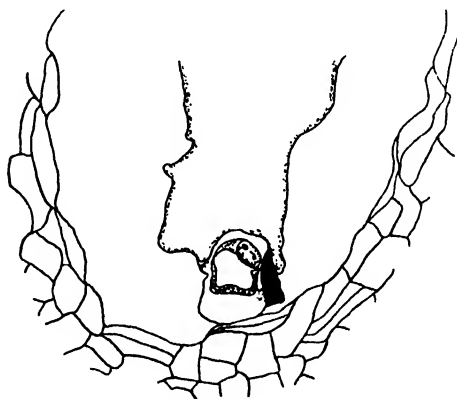


FIG. 15. Egg-cell about eighteen days after fertilization. $\times 540$.

embryo arose in the normal way from the fertilized egg. It consists of nine cells, and is surrounded by the original wall of the zygote.

The subsequent growth is for some time very slow. The youngest fruit in which an embryo could be detected by the naked eye was 10 cm. long, corresponding to an age of about eighty-seven days; and in this case the embryo when measured under the microscope proved to be only 0.2 mm. long. The embryos shown in Fig. 17 A and 17 B came from a fruit about

ten days older, whilst that shown at 17 C (3.5 mm. long) was one of the largest in a pod 1.5 cm. long, and probably at least 120 days old.

In the latter stages growth must be rapid, for at 130 to 150 days after fertilization the embryo is practically full-grown, and only traces of the jelly-like endosperm remain round the fleshy, wrinkled cotyledons.

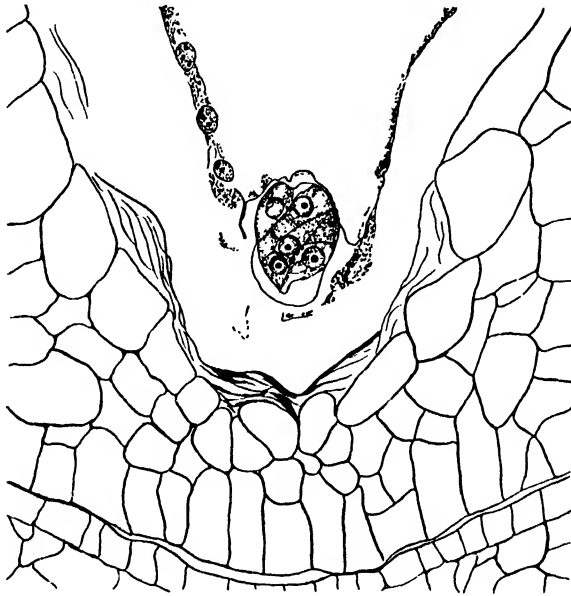


FIG. 16. Young embryo in a fruit about fifty days old. $\times 540$.

Parthenocarpy.

The occurrence of parthenocarpy in cacao has been suspected by several observers. The present work has afforded no evidence of such an occurrence, and whilst the possibility still cannot be denied, the writer is of the opinion that the idea arose from misinterpretation of stages in the normal embryogeny.

Wright (10, p. 55) says: 'Many of the fruits which die back first undergo a change in colour, usually becoming yellow or yellowish red, and finally shrivel and shrink until they are about one-fifth their original size; if such fruits are cut when the first indications of dying back appear, it will be noticed that though the position of each seed and its area is outlined, there are no signs of an embryo in most specimens. Such instances may be regarded as cases of succulence without fertilization. . . .' Against this observation it is sufficient to note that, as stated above, normal pods may be 10 cm. long before the embryos in them become macroscopic, whereas the critical stage at which fruits die back is usually much earlier, and it is very rare for a pod of such a size to fail in that manner.

Kuyper (*loc. cit.*) quotes von Faber as being of the opinion that parthenocarpny occurs in cacao, and himself adduces evidence which supports the opinion. Examining embryo-sacs which should have been fertilized, he found in the majority of them a degenerated mass in the place of the egg nucleus, whilst at the same time he could count as many as ten nuclei in the sac, and the nucellus was enlarging vigorously.

In Kuyper's preparations, however, the multiplication of the endosperm nuclei was correlated with separation of the nucellus from the integuments,

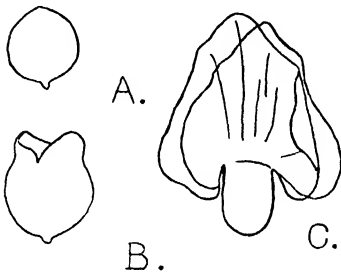


FIG. 17. Young embryos. A and B from a fruit about ninety days old. $\times 22$. C from a fruit about 120 days old. $\times 8$.

which he attributes to the latter growing faster than the former. It may be stated with some confidence that this appearance was due to the fixative used—absolute alcohol three parts, acetic acid one part. With that fixative the same appearance has been obtained in the present studies, but with Bouin's fluid it never occurred. Now Fig. 16 is evidence that even with Bouin's fluid there is strong distortion of the sac at this stage, and the egg-cell appears anything but vigorous, so that it seems quite possible that a fertilized egg-cell may have

been present in Kuyper's preparations, but was overlooked or mistaken for an endosperm nucleus.

Kuyper adds that in cases where he thought he observed traces of pollen-tubes the contents of the sac were richer, and the egg-cell better nourished, but as he admittedly never saw with certainty the entrance of any pollen-tube, little emphasis can be laid on this point. The present writer, on the contrary, would rather emphasize the fact that whilst minor differences do occur among the ovules of an ovary, no differences were ever observed by him sufficiently important to suggest that some ovaries, or some ovules of an ovary, were developing in a manner unlike that of the rest. Poverty of protoplasm is a characteristic of the sac from soon after fertilization until a fairly late stage in the development of the seed, and this, coupled with distortions consequent on fixation, may easily give rise to misleading appearances.

The Integuments.

Kuyper noted that in the young ovule the outer integument is developed on the outer side only, and there exceeds the inner, so that at fertilization the micropyle is formed by the outer side of the outer integument and the inner side of the inner (*cf.* Fig. 2). Later the outer side of the inner integument begins to grow; the outer integument is then pressed against

the endocarp, and its cells contain very little protoplasm, whilst later still Kuyper found it staining a deep black, whence he was led to conclude that it finally degenerates altogether. This, however, is not the case; it begins to grow again, and in the later stages of the seed is a much more prominent structure than the inner integument. The cells of its hypodermal layer on the outer side, and some of the next layer beneath, become prismatic in shape and highly mucilaginous, their walls ultimately disappearing, so that the ripe seed is surrounded by a continuous sheath of mucilage.

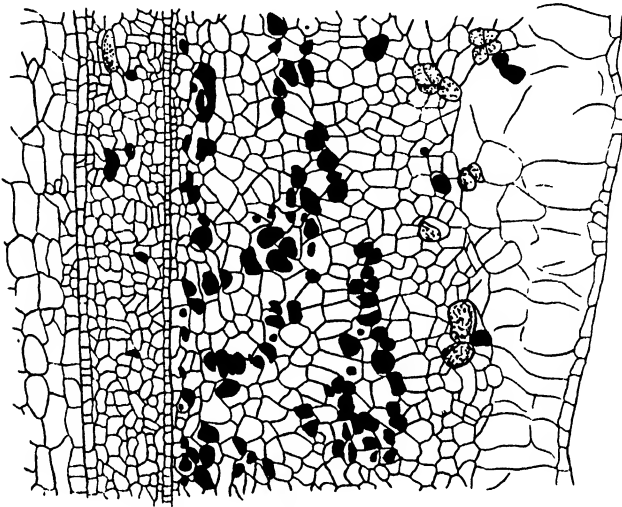


FIG. 18. Structure of the integuments, from the same young seed as in Fig. 13. Cells shaded black contain tannin. The tissue on the extreme left is perisperm. $\times 125$.

The relative size of the integuments in the half-grown seed can be seen by reference to Fig. 13, whilst Fig. 18 shows their structure at the same stage.

Another point of interest in the development of the seed is the behaviour of the cells in the region of the chalaza. On the third day after fertilization a few cells in this position stain a deep black, and the usual tests with iron salts and with potassium bichromate show that they are filled with tannin. A similar thing happens to a few cells in the vicinity of the micropyle. In the case of the chalazal cells the condition spreads until a large pad of tissue is involved (*cf.* Fig. 13); at the same time the cells often separate from each other and a resinous or gummy secretion fills the cavities between them. Since this pad of tissue lies directly in the fork of the vascular bundles supplying the outer integument, it must presumably affect the path of nutrients to the developing seed, preventing any direct passage of such into the base of the ovule.

Growth of the Fruit as a Whole.

When it was discovered that a pod which has almost attained its full size may contain embryos varying from 1 to 4 mm. in length, a series of measurements was started to show whether the subsequent development of the embryo is correlated with any peculiarity in the growth rate of the fruit as a whole.

For this purpose about fifty young pods of various sizes were marked and kept under observation. Their lengths and maximum diameters were

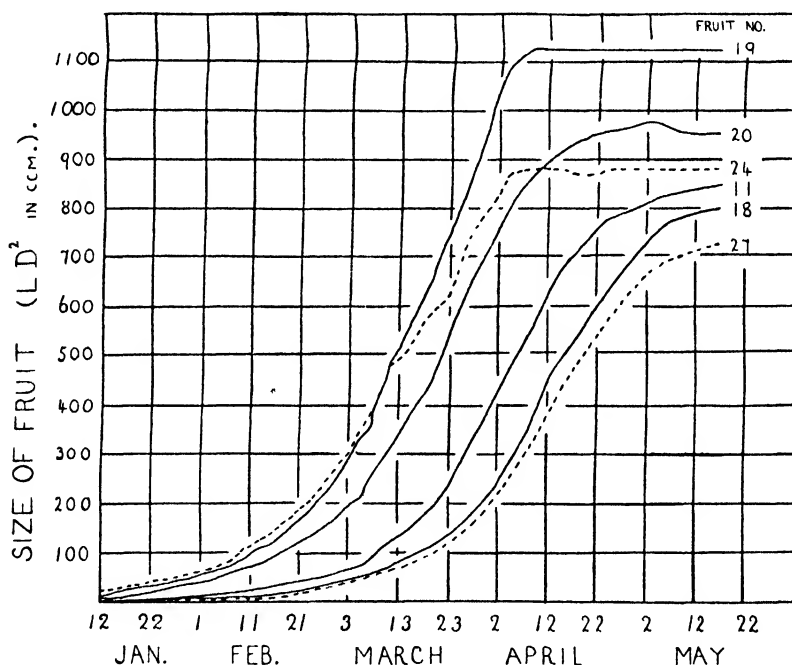


FIG. 19. Curves showing increase in size (length \times square of diameter) of six fruits on tree 125 from 12.1.25 to 17.5.25.

measured with vernier callipers every other day from 12.1.25 to 11.3.25, thereafter every fourth day until 12.4.25, and on several subsequent occasions, ending on 17.5.25, when twenty-four were surviving, of which nine were on one tree (No. 125). It may be mentioned in passing that nearly all the fruits used for investigating the later stages of the embryogeny were taken from this same tree, so that it was possible to form an approximate estimate of their age by measuring their length. Full-grown fruits from different trees vary so widely in size that figures given above for lengths and ages cannot be taken as of general application, but Fig. 20 shows that for the one tree during the course of the observations a reasonably accurate estimate could be made.

From these measurements the following facts were established :

1. The form of the growth curve is that usual for growth curves as a class, though some pods cease growing more abruptly than others. Fig. 19 shows curves for six fruits of tree 125, the value plotted being (length \times square of diameter). Fig. 20 gives the corresponding curves for increase in

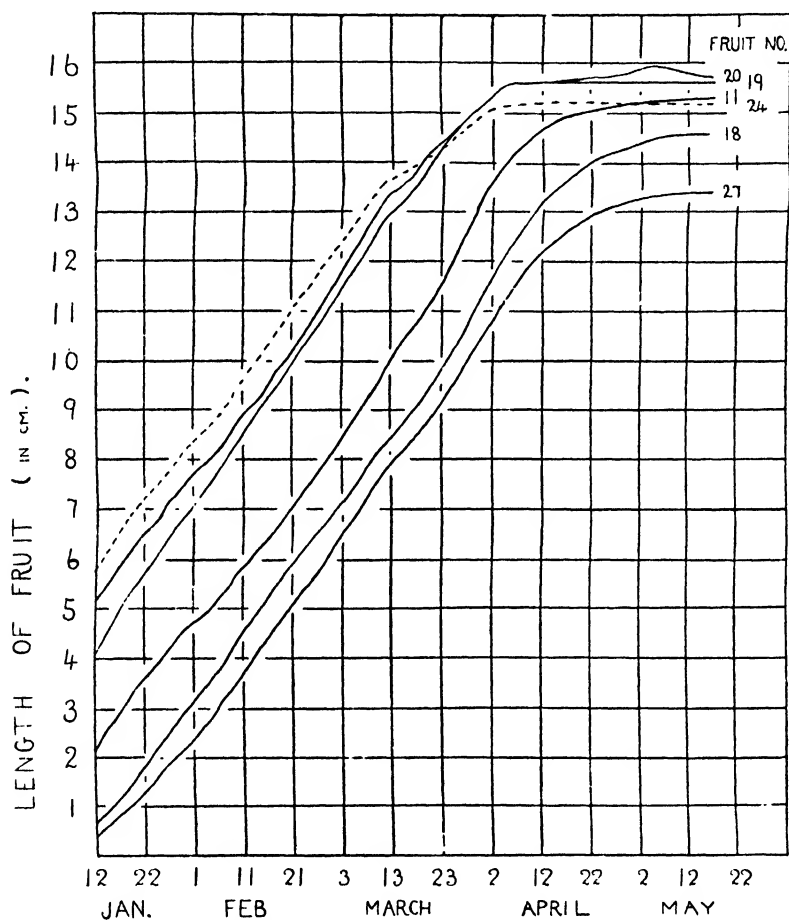


FIG. 20. Curves showing increase in length of six fruits on tree 125 from 12.1.25 to 17.5.25.

length alone. The latter appear to start as straight lines, but this is due to the fact that the youngest fruits were about ten days old when the measurements started—it being impossible to measure with any accuracy, or without damage, ovaries less than 5 mm. long.

2. Increase in the external dimensions ceases 130 to 150 days after fertilization, and if the pods are opened at this stage it is found that the embryos are almost full-grown with a very little endosperm still remaining. When this fact is combined with data given above, it appears that most of

the growth of the embryo must take place while that of the pod is declining to a standstill, but whether the two rates are interdependent or not cannot be stated.

It may be noted that these observations are contradictory to Hunter's statement (3, p. 170) that : 'das absolute Wachstum der Früchte ist jedoch in den ersten vier Monaten weniger stark als in den späteren Monaten'.

Blackening of Young Pods.

During the course of the experiment a number of the young pods under observation blackened and shrivelled, as young cacao pods so commonly do. In every such case it was noticed that growth ceased about four days before there was any sign of the approaching failure, and this cessation was followed by a slight decrease in both dimensions, soon after which browning became visible round the insertion of the stalk.

Growth of these fruits had previously been quite normal, and the course of events suggests a comparison with the abscission of cotton bolls, as investigated by Mason (5). Mason found that the growth-rate of the cotton boll falls normally into two distinct stages—a declining rate (independent of fertilization) for four to five days after anthesis, and after that an increasing rate in fertilized bolls, but in absence of fertilization a steady decrement, ending in shedding. He says : 'Negative growth-rates were generally shown by the boll for some one to three days prior to the completion of abscission. It was suggested that inability to secure the assimilates necessary for normal development diminished the water-absorbing power of the boll until a stage was reached at which the tension in the water column of the plant led to a suction of water from the boll. This, it seemed, was the cause not only of the negative growth-rates, but was actually the factor initiating abscission.'

That the failure of young cacao fruits is often due to malnutrition has been suggested by several observers. Wright (10), as noted above, thought that it might sometimes be due to lack of fertilization, but he adds : 'On the other hand, many cases have been observed where numerous and very large clusters of young cacao fruits have begun to set, but where the local food supplies appear to have been deficient, and dying back was inevitable.' Nowell (7) compares drying of young cacao fruits to boll-shedding in cotton, and considers it to be frequently 'an adjustment of numbers to the amount of fruit which can be brought to maturity'.

Further discussion of this question is outside the scope of the present paper, but it may be added that all the measurements were made in the dry season, and that the fruits showed themselves to be sensitive to changes in water-supply. It was noted on several occasions after a heavy shower that pods which had ceased growth some days previously showed a slight

increase in dimensions, which at the next measurement had disappeared again.

More extensive observations of the growth-rate of cacao pods should yield valuable information concerning the physiology of the plant.

SUMMARY.

1. Previous work by Kuyper on the development of the embryo-sac in *Theobroma Cacao*, and on the chromosome number of the species, has been confirmed. The development of the sac is normal.

2. Pollen-tubes have been traced to the micropyles which they reach in about four hours.

3. A normal process of fertilization was observed, and there is no reason to suppose that such is not the rule.

4. The endosperm has a prolonged free-nuclear stage, during which the nucellus enlarges. After wall-formation in the endosperm the perisperm is absorbed.

5. The egg-cell is tardy in division, but ultimately gives rise to a normal embryo. The occurrence of parthenocarpy in cacao, suspected by other workers, is very doubtful.

6. The embryo takes about ninety days to reach macroscopic size, and in nearly full-grown pods may be less than 2 mm. long, but its development is almost complete when the fruit stops growing.

7. The growth of the fruit as a whole was studied, and the failure of young pods to mature is briefly discussed in the light of observations made.

The writer wishes to record his thanks to Professor S. C. Harland for laboratory facilities freely afforded; to the Trinidad Department of Agriculture for the use of the cacao plot at St. Augustine Experiment Station; and to Drs. A. W. Hill and J. Kuyper for providing literature otherwise inaccessible.

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Chemical Studies in the Physiology of Apples.

VI. Correlation in the Individual Apple between the Mineral Constituents and other Properties.

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IN a former paper (2) of this series the author described preliminary work on the relationship between the mineral properties of apples and their environment (soil, season, and nature of stock). In that paper only the mean analytic data were given, but they were based on the analysis of thirty individual apples from the same orchard. In the present paper are given the full data of all the analyses. Furthermore, the data have been examined from another aspect, that of correlation of properties, in order to discover if there were any signs of relationship between the different constituents when the variable effects of soil and climate were eliminated by taking apples from the same environment. It was hoped that the results might help to explain the function of some of the mineral constituents, and their effect on the vital processes of the living plant.

Experimental.

Thirty Bramley's Seedling apples of the 1924 season, from a number of trees grown in the same orchard, were used for the experiment. The soil was alluvial gravel, and the trees were all mature, of approximately the same age, on vigorous stocks. The apples were selected at random from a large sample gathered on October 28. All appeared to be sound and in good condition. The apples were weighed and their colour was noted; they were cut up and dried on November 16, having been stored at 1° C. for three weeks. Of the mineral constituents, the total ash, phosphate, potash, and iron were estimated; other elements were omitted owing to the small amount of material in the individual apple. The nitrogen estimations were kindly undertaken by Miss Archbold (1); the acidity, density, and pH of the juice were also determined.

The methods employed were those already described by the author (2), with the slight modifications described below owing to limitation of material. The dry weight was found in the usual way, but the dried material was ground by hand in an agate mortar so as to prevent the introduction of any extraneous iron. The potash was determined by the perchlorate method, in the ash, from about 4 gm. of dried material, and sulphate was removed from the solution of the ash before the precipitation. The phosphate was estimated by the nephthelometer method: about 1 gm. of dried material was ashed, and the solution of the residue diluted to 250 c.c., of which 5-10 c.c. were used for each estimation. In the colorimetric determination of the iron, the concentration of the standard solution was suitably decreased; the test solution was made up from the ash of 2 gm. of dried apple dissolved in 100 c.c. of water and acid, 25 c.c. of which were used for each estimation. The pH of the juice was found electrically, using the apparatus described by Haynes (6). The acidity of weighed amounts of juice, varying from 3-6 gm., was found by titrating with N/10 sodium hydroxide, the volume being calculated from the densities.

Results.

The results of the analyses are shown in Table I. The mineral constituents are calculated as percentages both on the basis of the fresh weight of the cut-up apple (column B) and on that of dry weight (column A), and also of total ash (column C). The results were also referred to the original weights of the apples, but as no new relation of interest was shown, these figures have not been included in the table.

It is interesting to observe the connexion between mineral constitution and colour; differences are observed if green apples (1-7) are compared with yellow (18-22) and with red and yellow apples (22-6). The green apples appear to have lower mean values than the yellow or red and yellow for dry weight, total ash and potash, and pH value. The average weight of the green apples is also less, as might be expected, since the green apples were probably less ripe, and had not reached their full growth. The average content of phosphate in the green and in the yellow apples is about the same, though the percentage of phosphate in the total ash tends to be lower in the yellow, and red and yellow, apples. The nitrogen content is higher in the green apples, even when calculated on the basis of the original weight of the apple; this is in keeping with the high value for nitrogen previously found in immature Lane's Prince Albert apples (2). The iron content also tends to be slightly higher in the green apples.

The two apples suffering from bitter pit have higher total ash values

than the mean, and rather low percentages of phosphate, but the number of apples is insufficient for any inferences to be drawn, though the results agree with the estimations of apples suffering from bitter pit made by McAlpine (12) and by the author (2).

Other possible relationships between the properties were sought for by plotting the numerical values of the properties to be compared. As judged from these graphs the following properties showed very definite correlation :

Potash and total ash.

Phosphate and total ash.

Hydrogen-ion concentration and potash.

Hydrogen-ion concentration and total ash.

The correlation coefficient between hydrogen-ion concentration and potash was found to be -0.4755 , and between pH and total ash -0.4949 ; both of these values are significant. It appeared from the graphs that the other two correlation coefficients would have approximately the same value, so that it was unnecessary to calculate them.

Correlation coefficients were also calculated when the graphs showed only slight indications of relationship. Such calculations were made for the various pairs of the following: nitrogen, potash, phosphate, iron, and the acidity of the juice. Some of these constituents gave indications of varying directly, and some inversely, with one another, so it was obvious that correlations between some of the properties might be masked by the effects of the other variables. It was thus necessary to calculate the partial correlations—that is, correlations eliminating the effect of other variables on the properties to be compared. These calculations are very tedious, but the results, as shown in Table II, indicate that it was worth the expenditure of time, for when three variables are eliminated, several pairs are shown to be correlated that were not obviously related from a study of their graphs.

The amount of calculation that would be required to work out the partial correlations between all the ten variables was far too large to be undertaken, but the five variables selected were those which seemed likely to be most fruitful. Adding to the number of variables would have altered very slightly the value of the partial correlation coefficients for the five variables. Between two factors *A* and *B* the elimination of the effect of the variable *C* only appreciably affects the correlation coefficients if the values of *A* with *C* and *B* with *C* are appreciable and of opposite signs. If one of the relations *A* and *B*, or *B* and *C*, is almost zero, very little change is observed when *C* is eliminated from the relation *A* and *B*; for this reason elimination of the variables of total ash, dry weight, pH, and density would have made very little difference to the significance of the results in Table II.

TABLE I. *Results of Analyses of 30 Bramley's Seedling*

Figures in Column A represent percentage referred to dry weight.

" " B " " " fresh weight.

" " C " " " total ash.

Condition of Apple.	Weight of Apple in grm.	Dry Weight. B	Total Ash.		Potash (K_2O).			Phosphate (P_2O_5).		
			A	B	A	B	C	A	B	C
1. Green . . .	154	11.17	2.076	0.2319	1.052	0.1175	50.7	0.247	0.0275	11.89
2. " . . .	157	10.77	2.088	0.2242	1.087	0.1170	52.1	0.230	0.0248	11.08
3. " . . .	162	10.66	2.171	0.2317	1.096	0.1167	50.1	0.176	0.0187	8.09
4. " . . .	159	10.64	1.974	0.2100	1.029	0.1094	52.1	0.184	0.0196	9.35
5. " . . .	165	11.25	1.703	0.1917	0.875	0.0969	50.5	0.165	0.0186	9.66
6. " . . .	160	11.01	1.746	0.1920	0.878	0.0965	50.3	0.182	0.0200	10.41
7. " . . .	162	10.98	1.931	0.2120	1.020	0.1120	52.9	0.199	0.0218	10.31
8. Green and red . .	188	11.34	1.853	0.2102	0.935	0.1060	50.4	0.152	0.0172	8.14
9. " " . .	175	11.88	1.700	0.2020	0.903	0.1074	53.2	0.157	0.0186	9.22
10. Green, slightly red .	194	10.77	1.954	0.2100	1.210	0.1303	62.0	0.222	0.0238	11.31
11. Do., one scab . .	213	11.28	2.025	0.2280	1.019	0.1148	50.4	0.159	0.0180	7.90
12. Red . . .	173	10.90	2.070	0.2259	1.054	0.1150	50.9	0.184	0.0200	8.85
13. Medium . . .	177	12.59	1.781	0.2240	0.966	0.1216	54.2	0.167	0.0210	9.38
14. Green, yellow, and red	185	11.04	1.950	0.2150	1.140	0.1260	58.6	0.118	0.0131	6.10
15. Green and yellow .	189	11.51	2.540	0.2922	1.468	0.1690	57.8	0.180	0.0207	7.10
16. " " . .	182	10.96	2.256	0.2470	1.239	0.1357	54.9	0.207	0.0226	9.17
17. " " . .	179	11.41	1.936	0.2210	1.065	0.1215	55.0	0.195	0.0224	10.11
18. Yellow and red . .	153	11.78	1.720	0.2017	0.850	0.1000	49.2	0.115	0.0135	6.73
19. " " . .	150	11.95	2.136	0.2550	1.218	0.1455	56.0	0.226	0.0274	10.64
20. " " . .	183	12.08	1.901	0.2297	1.136	0.1371	59.5	0.141	0.0170	7.41
21. " " . .	167	12.15	2.230	0.2705	1.318	0.1600	59.2	0.225	0.0274	10.11
22. Yellow . . .	173	11.26	2.266	0.2552	1.229	0.1381	54.2	0.203	0.0228	8.95
23. " . . .	158	10.97	1.977	0.2170	1.054	0.1155	53.2	0.187	0.0205	9.45
24. " . . .	183	12.12	2.670	0.3240	1.455	0.1762	54.5	0.232	0.0281	8.69
25. " . . .	186	12.28	1.990	0.2440	1.102	0.1353	55.5	0.164	0.0202	8.26
26. " . . .	175	10.64	2.352	0.2500	1.239	0.1319	52.7	0.193	0.0206	8.23
27. " scab . .	209	11.60	1.725	0.2001	0.972	0.1128	56.8	0.102	0.0118	5.93
28. Slight bitter pit .	170	10.86	2.209	0.2396	1.382	0.1500	62.6	0.183	0.0198	8.39
29. Bitter pit, yellow .	187	12.63	2.297	0.2900	1.289	0.1629	56.1	0.187	0.0236	8.15
30. Bitter pit, slightly red.	175	11.45	2.170	0.2484	1.059	0.1211	49.2	0.156	0.0178	7.15
Mean values . . .	175	11.40	2.048	0.2324	1.107	0.1269	54.2	0.181	0.0206	8.54

Apples from Canterbury (Gravel Soil) (Nov. 1924).

Figures in Column A represent percentage referred to dry weight.

" " B " " " fresh weight.

" " C " " " total ash.

Condition of Apple.	Iron (F_2O_3).			Nitrogen.		Properties of the Juice.		
	A	B	C	A	B	Density.	Acidity N.	pH.
1. Green . . .	0.00383	0.000426	0.184	0.530	0.0591	1.0509	0.199	2.95
2. " . . .	0.00333	0.000358	0.160	0.424	0.0456	1.0483	0.193	2.99
3. " . . .	0.00363	0.000386	0.167	0.554	0.0590	1.0534	0.228	2.87
4. " . . .	0.00419	0.000445	0.212	0.441	0.0470	1.0543	0.208	2.88
5. " . . .	0.00401	0.000451	0.236	0.320	0.0360	1.0519	0.187	2.89
6. " . . .	0.00509	0.000560	0.292	0.602	0.0663	1.0523	0.170	2.89
7. " . . .	0.00356	0.000390	0.184	0.435	0.0477	1.0477	0.170	2.95
8. Green and red . .	0.00419	0.000475	0.226	0.395	0.0448	1.0517	0.188	2.95
9. " " . . .	0.00356	0.000422	0.209	0.240	0.0285	1.0568	0.188	2.90
10. Green, slightly red .	0.00388	0.000416	0.198	0.460	0.0495	1.0481	0.172	2.95
11. Do., one scab . .	0.00255	0.000287	0.126	0.270	0.0304	1.0523	0.191	3.05
12. Red . . .	0.00249	0.000271	0.120	0.179	0.0195	1.0502	0.195	2.98
13. Medium . . .	0.00392	0.000494	0.220	0.382	0.0480	1.0542	0.185	3.09
14. Green, yellow, and red	0.00293	0.000324	0.151	0.311	0.0344	1.0509	0.145	2.92
15. Green and yellow .	0.00307	0.000353	0.121	0.409	0.0471	1.0562	0.174	3.06
16. " " . . .	0.00399	0.000437	0.177	0.457	0.0501	1.0522	0.198	2.93
17. " " . . .	0.00389	0.000444	0.201	0.286	0.0326	1.0482	0.164	3.05
18. Yellow and red . .	0.00299	0.000352	0.174	0.193	0.0227	1.0586	0.167	3.01
19. " " . . .	0.00388	0.000464	0.182	0.225	0.0266	1.0542	0.181	3.02
20. " " . . .	0.00364	0.000439	0.191	0.153	0.0185	1.0500	0.137	3.07
21. " " . . .	0.00263	0.000319	0.118	0.267	0.0324	1.0614	0.166	3.02
22. Yellow . . .	0.00322	0.000362	0.142	0.583	0.0655	1.0507	0.196	2.96
23. " . . .	0.00379	0.000415	0.191	0.232	0.0254	1.0490	—	2.89
24. " . . .	0.00280	0.000340	0.105	0.255	0.0306	1.0619	0.216	3.02
25. " . . .	0.00669	0.000820	0.336	0.472	0.0580	1.0535	0.171	3.05
26. " . . .	0.00456	0.000485	0.194	0.212	0.0225	1.0538	0.185	3.02
27. " scab . . .	0.00289	0.000334	0.167	0.518	0.0601	1.0554	0.152	3.02
28. Slight bitter pit . .	0.00251	0.000272	0.114	0.399	0.0433	1.0497	0.153	3.01
29. Bitter pit, yellow .	0.00291	0.000367	0.127	0.184	0.0232	1.0544	0.173	3.06
30. Bitter pit, slightly red .	0.00352	0.000404	0.162	0.334	0.0382	1.0502	0.152	3.13
Mean values . . .	0.00340	0.000396	0.174	0.357	0.0404	1.0527	0.179	2.986

The calculations have been made in the usual way (3). The following formulæ were used :

$$r_{AB} = \frac{\Sigma xy}{\sqrt{\Sigma x^2 \Sigma y^2}} \quad \text{and} \quad r_{AB.C} = \frac{r_{AB} - r_{AC} \times r_{BC}}{\sqrt{(1 - r_{AC})(1 - r_{BC})}}.$$

r = correlation coefficient.

A , B , and C = variables.

Σx^2 (or Σy^2) = sums of the squares of the differences from the mean.

Σxy = sums of the products of the differences from the means of the two variables.

The significance of the results has been found by reference to Fisher's table, whence it appears that, for the number of apples used in the experiment, a value of r greater than about 0.31 is significant.

Of the correlations apparent from the graphs (p. 130), that between potash and total ash is to be expected, since potash constitutes more than 50 per cent. of the ash. The correlation of phosphate with total ash is considerably less; this correlation is also probably due to the fact that a considerable proportion of the ash (about 10 per cent.) consists of phosphate. The reduction in hydrogen-ion concentration by total ash and potash is a buffer effect; it is chiefly the potash content of the ash, in the form of potassium malate, which drives back the ionization of the malic acid.

One of the chief points of interest shown in Table II is that the nitrogen of the apple shows no correlation with any of the other properties determined. In the leaves of the vine Lagatu and Maume (9) found a linear relationship between the nitrogen and phosphorus content, and it is stated by Gardner, Bradford, and Hooker (5) that analyses of the trunk, branches, and 'new growth' of fruit trees show the same linear relationship, indicating that the nitrogen and phosphorus are present in the same molecule. Since nucleic acid and lecithin and allied substances contain both these elements, these authors considered that the bulk of the phosphorus in the organs was present in organic form in lecithins and nucleins. However, in the apple itself no relationship was found between the nitrogen and phosphorus; this indicates that a fleshy organ like the apple, with a low protoplasmic content, has only a small percentage of its phosphorus in organic form. That this is probably the explanation has been shown by the author in some preliminary unpublished work on the phosphate compounds in the apple. It was found that about 70–80 per cent. of the phosphate present consisted of free mineral phosphates and hexose-phosphates. This is in agreement with analyses of straw by Nanji and Shaw (13), who found that 60–70 per cent. of the phosphate present was acid-soluble. It is interesting that, in analyses of the soil, Potter and Benton (14) found that a similar percentage of inorganic phosphate was

present, leaving about 30 per cent. in organic form. In a preliminary experiment the author found that only about 12 per cent. of the phosphate in apples was in the form of lecithin. Thus it is probably owing to the presence of large and variable proportions of nitrogen-free phosphorus compounds that no correlation is to be found in the apple between these two elements.

TABLE II. *Values of Partial Correlation Coefficients. (The symbols in brackets represent the variables that have been eliminated.)*

<i>Properties compared.</i>	<i>Correlation Coefficient.</i>	<i>Eliminating 1 Variable.</i>	<i>Eliminating 2 Variables.</i>	<i>Eliminating 3 Variables.</i>	<i>Value of p.</i>
<i>Significant.</i>					
Phosphate and potash .	+0.50362	+0.52255 (N ₂) +0.57594 (Fe ₂ O ₃)	+0.59165 (N ₂ and Fe ₂ O ₃)	+0.6627 (N ₂ , Fe ₂ O ₃ , and acid)	p<0.01
Phosphate and iron .	+0.09591	+0.09209 (N ₂)	+0.33750 (K ₂ O and N ₂)	+0.3585 (K ₂ O, N ₂ , and acid)	p=0.5
Phosphate and acidity .	+0.42227	+0.53411 (K ₂ O) +0.41913 (N ₂)	+0.46545 (N ₂ and K ₂ O) +0.41637 (N ₂ and Fe ₂ O ₃)	+0.5355 (K ₂ O, N ₂ , and Fe ₂ O ₃)	p<0.01
Potash and acidity .	-0.07515	-0.04336 (N ₂)	-0.33866 (N ₂ and P ₂ O ₅) -0.02570 (N ₂ and Fe ₂ O ₃)	-0.3712 (P ₂ O ₅ , N ₂ , and Fe ₂ O ₃)	p=0.5
Potash and iron . .	-0.34649	-0.34058 (N ₂)	-0.45713 (N ₂ and P ₂ O ₅)	*	p<0.01
<i>Non-significant.</i>					
Nitrogen and potash .	-0.16939	-0.23234 (P ₂ O ₅) -0.15565 (Fe ₂ O ₃)	-0.22945 (Fe ₂ O ₃ and P ₂ O ₅)	*	p>1
Nitrogen and phosphate	+0.06147	+0.17247 (K ₂ O) +0.05529 (Fe ₂ O ₃)	*	*	p>1
Nitrogen and iron .	+0.06848	*	*	*	p>1
Nitrogen and acidity .	+0.19624	*	*	*	p>1
Acidity and iron . .	+0.06871	+0.05649 (N ₂)	+0.01979 (N ₂ and P ₂ O ₅) +0.04420 (N ₂ and K ₂ O)	*	p>1

The values are calculated from the figures in the B columns of Table I (percentages referred to fresh weight).

* Elimination of further variables would have no effect on the significance of the result.

According to Archbold (1), all the nitrogen present in the apple is in organic form (protein), and the work of Kostytchew and Eliasberg (7) has shown that in plants the potash is all present in inorganic form, since it can

be completely extracted with water. Potash must therefore exist chiefly as phosphates, sulphates, and salts of organic acids. Thus no correlation between nitrogen and potash is to be expected on the simple ground of chemical combination. There might, however, be some relation between potash and protein (of which nitrogen is a measure) if, as has been stated by Weevers (22 and 23), potash is essential for protein formation. According to Loew (10) potash acts as a condensing agent, and is necessary for both carbohydrate and protein formation, and he noted that in seeds there is a closer relation between potash and protein than between potash and starch. But no such positive relation was found in the apple, there being, however, a slight indication of a negative relationship. This may merely mean that the correlation between potash and protein does not exist in the apple, which contains only a very low percentage of protein, and is a senescent organ.

The results of Table II, however, are slightly more in favour of the importance of potash in carbohydrate formation. This claim is possibly supported by Stoklasa (18) and Stoklasa and Senft (19), who found that sugars can be synthesized from carbon dioxide and water in the presence of sunlight and potassium hydroxide. Sorauer (17) is also in favour of this theory of the function of potash in the plant, and states that in carbohydrate storage organs the amount of carbohydrate formed is directly dependent on the supply of potash present. The figures for the dry weight in Table I may be taken as an approximate measure of carbohydrate content, and it will be seen that there is some indication of a direct correlation between carbohydrate and potash, though it is not sufficiently large for certainty. However, the fact that the apple contains a large quantity of sugar and also a higher percentage of potash than any other part of the tree is an indication that there is some relation between the substances. Cultural work on the absence and shortage of potash has been carried out by many investigators, and on the whole appears to support the theory that potash is primarily essential for carbohydrate formation, but is also necessary for protein-synthesis. Weizmann (24) found that a potash-starved plant loses its power of synthesis, and the reserve storage organs are affected; though the number of leaves and shoots is unaltered, their size and development are more and more retarded until the foliage dies. Smith and Butler (16) found the same dwarfing of the axis and death of foliage, but considered that their results supported the theory of the influence of potash on protein, rather than on carbohydrate assimilation. Wallace (20) and Mann (11) found that the absence of potash caused restricted root growth, and the leaves were susceptible to 'scorch'. This may be due to the importance of potash in assimilation, so that when the supply is low the plant is undernourished and possibly more susceptible to scorch.

Of the significant correlations, that between potash and phosphate has

the highest value. This is to be expected, since a large proportion of the phosphate is present either as free mineral phosphate or as hexose-phosphate, and therefore combined with a metallic radical which would tend to be potash, as potash is the chief basic element present. That the correlation is not higher is due to the fact that some of the phosphate, as stated above, is in the form of lecithin and unassociated with potash.

The correlation between phosphate and iron may be related to the association of both these elements with respiration. Iron is supposed to be essential for respiration, and if carbohydrate respiration in plants is similar to alcoholic fermentation, as Raymond (15) has suggested of animals, then phosphates would also take a vital part in the process. Warburg (21) states that if an inorganic phosphate and fructose are present in aqueous solution a substance is formed which combines with iron, changing it into a catalytically active form; this reacts quickly with molecular oxygen, giving iron in a higher state of oxidation, which reacts with the organic substance—in this case the sugar—and is reduced again to bivalent iron. He found that oxygen disappears when passed through a neutral solution of fructose and sodium phosphate containing iron, but this does not occur if any other salt is substituted for phosphate, and is very slow with all sugars except fructose. Since fructose is the chief sugar present in apples, it is possible that in respiration a similar process is taking place, and the correlation in the fruit between the phosphate and iron may be thus explained. That the correlation is no greater may be due to the fact that only free mineral phosphate and iron are related; thus the varying weights of lecithin, and possibly of hexose-phosphates also, render the correlation lower between total phosphate and iron.

The correlation between phosphate and acidity is very high; this may be associated with the effect of phosphates on the oxidation of the sugars, as acids are among the first oxidation products of respiration. During the rapid fermentation of yeast in the presence of phosphates, succinic, malic, acetic, oxalic, and other acids are formed in various proportions and, according to Kostytschew and Frey (8), a similar process accounts for the presence of these acids in plants. The acids which were found by Franzen and Helvert (4) to be present in apples are malic, citric, and succinic, also traces of lactic, oxalic, and unsaturated acids.

There is a significant though not a very large relationship between potash and acidity, a high potash value tending to be associated with a low acidity. Organic acids are formed as respiration products, but in the presence of greater amounts of potash, possibly more of their potassium salts are produced, this leaving less acid as the free organic acid.

SUMMARY.

The dry weight, total ash, potash, phosphate, iron, nitrogen, and also the density, acidity, and pH of the juice, were determined in each of thirty Bramley's Seedling apples from the same orchard.

The green apples analysed show lower percentages of dry weight, total ash, potash, and pH, and a higher percentage of nitrogen when compared with the yellow and the red and yellow apples.

Low values for the hydrogen-ion concentration of the juice were found to be associated with high total ash and potash content.

The third partial correlations were calculated between potash, phosphate, iron, nitrogen, and acidity. Significant values, showing direct correlation between the elements compared, were obtained for potash and phosphate, phosphate and acidity, and phosphate and iron. Potash and iron, and potash and acidity, are inversely correlated. No significant correlations between nitrogen and any of the mineral constituents are to be observed.

Suggestions are made to account for the observed correlations.

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The Life-history of *Padina Pavonia*.

I. The Structure and Cytology of the Tetrasporangial Plant.

BY

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With Plates VIII and IX and four Figures in the Text.

INTRODUCTION.

PADINA PAVONIA is a Mediterranean member of the Dictyotaceae. Its distribution around Great Britain is limited to certain isolated localities on the south coast of England, roughly from Hampshire to Cornwall. The asexual (sporophyte) and monoecious sexual (gametophyte) plants do not differ in external appearance, both having the form of fan-shaped thalli. Many algologists have observed that whilst the tetrasporic plants are abundant in certain localities, the plants bearing sexual organs are extremely rare. The writer examined some hundreds of plants during the summers of 1924 and 1925, but did not collect a single sexual plant. This disparity also obtains in Mediterranean waters.

Church (3), writing on the polymorphy of *Cutleria multifida*, discusses the difference in distribution of *Cutleria* and *Aglaosonia* and submits evidence to show that temperature is the most important factor determining the occurrence of these plants. It is not difficult to imagine that temperature and other external factors contribute largely to variations in distribution of such plants as *Cutleria* and *Aglaosonia*, which differ from each other morphologically. In the case of *Padina*, however, the tetrasporic and sexual plants are of the same form and size. Considering that the tetraspores are produced in large numbers, and that they germinate very readily, it would therefore appear that the inequality in distribution must be attributed to some inherent quality in the constitution of the two forms of plants.

With this striking inequality in numbers of the tetrasporic and sexual plants of *Padina* in mind, Professor Lloyd Williams suggested that a

complete cytological study of all forms of this alga might throw light on its peculiar behaviour. This paper forms a part of that investigation, but deals exclusively with the tetrasporic plant, and mainly with the nuclear changes in the tetraspore mother-cell.

HISTORICAL.

Nägeli (14) in 1847 described the growth of the fan-shaped thallus as taking place by means of a series of marginal cells, but it remained for Reinke (15) in 1878 to describe the morphology of the plant. He drew attention to the three kinds of branches, which he terms 'Rundtriebe' (cylindrical in form), 'Flachtriebe' (the flat growths), and 'Breittriebe' (the fan-shaped thallus). Before this time the Rund- and Flachtriebe seem to have escaped notice, and all references, including that of Nägeli, were to the fan-shaped 'Breittriebe'. The 'Rundtriebe' and 'Flachtriebe' resemble each other closely, both having an apical form of growth, and Reinke shows the connexion between this apical growth and the marginal growth of the fan-shaped thallus. Bitter (2) contributed a paper describing the effect of illumination on the production of the hair-like outgrowths and the rolling of the margin. Richards (16) compared *Zonaria* with *Padina Pavonia* and other members of the Dictyotaceae.

Wolfe (20), working on *Padina variegata*, carried out a series of cultural experiments in the sea, and proved that there is a definite alternation of generations in this dioecious species, and concluded that whilst there is parthenogenetic germination, the plants resulting from these unfertilized eggs do not reach maturity and develop any fruiting bodies. No cytological investigations are recorded. The earliest reference to the cytology of *Padina Pavonia* which I have been able to trace occurs in the 'Studies in the Dictyotaceae. I' by Lloyd Williams (18). This work describes the cytology of the tetrasporangium of *Dictyota dichotoma*, but frequent references are made to, and comparisons drawn with, *Padina Pavonia*. As will be shown, the nuclear changes occurring in the tetrasporangium of *Padina* are, in the main, very similar to those already described for *Dictyota*. Georgevitch (6) in two very brief articles outlined the development of both the sexual organs and the tetrasporangia of *Padina Pavonia*, but his account is not illustrated. Except in a few points to be mentioned later my results are in general agreement with his recorded researches.

METHODS.

This investigation was begun on material kindly supplied by Professor Lloyd Williams and collected by him at Ladram Bay, in Devon. The bulk of the material used was collected by the writer during August, 1924, and the summer of 1925 on the Dorset coast. Plants were collected at Chapman's Pool, near Swanage, at Lulworth Cove, and on the Nothe rocks at

Weymouth. The alga is fairly abundant on these rocks, which face SSE. and are sheltered on the northern side by the elevated Nothe gardens. It flourishes in sandy rock pools which are exposed only at low water of spring tides, and are composed of stiff blue Oxford clay, which outcrops in several places in this neighbourhood (7), overlaid by a few inches of sand. It occurred to the writer that possibly there is some chemical ingredient of this blue clay which accounts for the abundance of *Padina* in this habitat.

Additional material of both tetrasporic and sexual plants was obtained from the Marine Biological Station at Naples through the courtesy of the Director and Professor Funk.

Material was fixed at various times of the day, but mainly at low water, the time of collecting. The material to be fixed during the night was kept in sea-water which was frequently changed. Various strengths of fixative were experimented with, the most satisfactory being Flemming's stronger solution diluted at the time of fixing with filtered sea-water to one-third strength. The material forwarded from Naples was, at my request, in two lots; one fixed in Flemming's mixture diluted to one-third strength, the other fixed in Flemming's made with sea-water as used by me. There is some evidence, not yet conclusive, that material fixed in the latter fixative is better. The best results were obtained after fixing for about six hours and then washing in sea-water. Slides were prepared with sections cut in three different directions, viz. some were cut transversely to the concentric zones, others tangentially to these zones, with a third series cut parallel to the surface of the thallus. Sections were cut at thicknesses varying from 4μ to 10μ , the thicker ones giving valuable information as to the form and arrangement of the tissues. Most of the drawings are from sections cut at 4μ or 6μ . Some of the material was bleached with hydrogen peroxide during the 70 per cent. stage of dehydration before embedding. In other cases bleaching was done on the slide, using either hydrogen peroxide, chromic acid, or chlorine. All gave satisfactory results. Various stains were used. Heidenhain's iron-alum-haematoxylin was employed both with and without Congo red or orange G as counter-stains. The combination with orange G gave some excellent results, especially during the prophase stages of the heterotypic division. Brazilin as a basic dye, with picric-acid Hoffmann blue as a counter-stain, is useful in the earlier stages when the tetrasporangium is young. Flemming's triple stain gave some interesting results.

The germination of the tetraspores was kept under observation in culture dishes containing filtered sea-water. One series of cultures was left undisturbed, apart from adding water to replace that lost by evaporation. Miquel's culture solution was added to a second series, whilst the sea-water in a third series was changed once a month. The results so far obtained do not warrant conclusions being drawn as to the relative merits of these

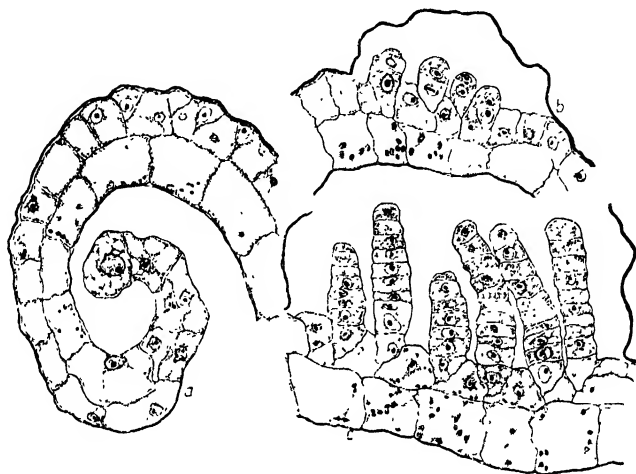
different cultural methods. An automatic tidal apparatus with aerating arrangements was also employed. This will be described later. Cover-slips to which the germinating tetraspores had attached themselves were removed periodically from the cultures, fixed, stained, and mounted. Flemming's solution (diluted) and acetic-carmin were both employed. The latter fluid fixes and stains at the same time.

MODE OF GROWTH AND ORIGIN OF 'HAIRS'.

In the fan-shaped thallus ('Breittriebe') of both tetrasporic and sexual plants, growth takes place by the active division of a row of marginal cells. This row really represents a row of apical cells, and so the mode of growth, in being a form of apical growth, is not unlike that in *Dictyota*. These meristematic cells are brick-shaped, with their outer (distal) walls curved and their long axes placed at right angles to the margin of the thallus. Cells are cut off from these apical cells by transverse walls, and very shortly afterwards these newly cut-off cells divide tangentially into two daughter-cells. Of these two cells, the dorsal one now elongates much more rapidly than the ventral one, and thus the margin of the thallus gradually curves over on itself and the characteristic inrolled condition is attained. This rolling up of the margin provides excellent protection for the tender cells of the formative edge.

For some distance from the apex the thallus is but two layers thick, but farther back the ventral cell is seen to have cut off a smaller surface cell with the result that the thallus attains a thickness of three cells. A characteristic feature of *Padina* is the hairs which beset both surfaces of the thallus, though they are more abundant on the dorsal surface. They occur in prominent and concentric bands and are developed as outgrowths of special superficial cells. Text-fig. 1 shows three stages in the development of these hairs. They originate in that portion of the thallus which is still rolled up. Text-fig. 1, *a*, shows the position of their origin, but does not indicate that they are protected by still another roll of the frond. The cells which give rise to hairs possess large nuclei, and the cytoplasm is much denser than that of neighbouring cells. The nucleus divides and a daughter nucleus is shut off by an oblique wall. This small cell thus cut off is filled with dense cytoplasm, and the nucleus enlarges until it occupies the greater part of the cell cavity. From four to eight adjacent cells undergo this process about the same time, so that the zone of hairs is composed of several rows. Almost invariably the wedge-shaped cell is cut off from the distal side of each hair mother-cell. Thus the suggestion conveyed by some published figures that the hairs are outgrowths of entire superficial cells is an erroneous one. By further cell division these protuberances become short rows of cells, each with a large nucleus. The nuclei in these hair-cells are very much larger than those in the ordinary cells of

the thallus. As first pointed out by Nägeli (14) these rows of hairs are at first covered by a common cuticle (Text-fig. 1, *b*), which is ruptured as the hairs increase in length (Text-fig. 1, *c*). As growth proceeds and the portion of the thallus bearing these hairs unrolls, they continue to increase in length until, when they come to lie on the flat dorsal surface they may be several centimetres in length. In the older parts of the thallus they are broken off.



TEXT-FIG. 1. *a*. A portion of the rolled margin of the fan-shaped thallus in longitudinal section, showing the hair rudiments cut off by oblique walls. These cells possess abundant cytoplasm and large nuclei. *b*. The hair rudiments beginning to grow out and lifting the common cuticle. Mitosis is seen in one of the young hairs. *c*. A later stage with the hairs now seen as filaments of small cells containing large nuclei. The cuticle has been ruptured. $\times 300$.

These zones of hairs mark the positions of the origin of the reproductive cells, whether sexual or asexual, and, among other functions, they serve in preventing particles of sand from settling down on the surface of the thallus.

THE EARLY DEVELOPMENT OF THE TETRASPORANGIUM.

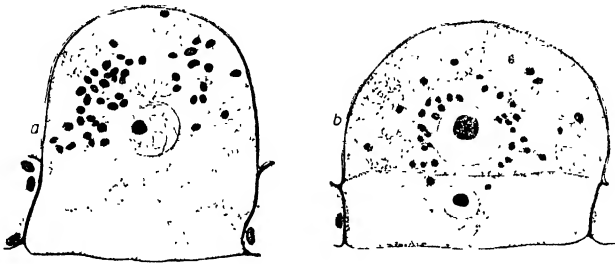
At first sight it appears that the regular arrangement of the reproductive bodies into concentric zones would make the fan-shaped thallus an object of easy cytological study. Generally speaking it is found that contiguous tetrasporangia in the various zones are at about the same stage of development. In the band nearest to the inrolled margin of the thallus they are found to be at, or near, stalk-cell division; whilst in the next zone nearer the base most of them possess nuclei in synapsis, or in some later stage of meiotic division. A still older band might contain fully developed tetraspores. In the older zones young sporangia are also sometimes initiated, but these belated members were not utilized in studying critical nuclear stages. In spite of this apparent regularity the suggestion of an

easy solution to the various nuclear stages which is given by a superficial survey is not altogether fulfilled on investigation. In the first place, the nuclei are small and the number of chromosomes is large. Matters are further complicated by the rapidity with which some critical stages are passed through, as well as by certain interesting peculiarities and abnormalities which will be described later.

In healthy plants the tetrasporangia occupy a zone of several rows on either side of the zone of hairs. The development of the tetrasporangium is very similar to that obtaining in *Dictyota*. One of the superficial cells on the dorsal surface enlarges, at first laterally and outward, until it protrudes to a considerable extent; its length being three or four times its width and the diameter of the top greater than that of the base. This process occurs in a number of superficial cells lying side by side, thus giving the concentric zones of tetrasporangia on the thallus. During this process the bulk of the cytoplasm containing the plastids passes towards the outer convex wall, and large vacuoles appear in the basal portion (Text-fig. 2, *a*). At this stage in development there are no sharply defined zones in the cytoplasm, and the chromoplasts, which are distributed fairly evenly throughout the tetrasporangial rudiment, are much more numerous than in the ordinary superficial cells of the thallus. On account of their rapid multiplication during the subsequent growth of the tetrasporangium they are much smaller than those of the vegetative cells. They are of different forms, either spherical or elliptical, or frequently bean-shaped. The elliptical nucleus of this tetrasporangial rudiment lies at first with its long axis parallel to the surface of the thallus, but soon it assumes a position at right angles to this. It enlarges considerably, a definite chromatic reticulum becomes evident, and the nucleolus stains deeply with basic dyes.

As the tetrasporangial rudiment continues to enlarge, a definite spireme forms in the nucleus, and a very faint centrosome with centrosphere is seen at the distal pole. As in *Dictyota*, *Sphacelaria*, and other types, this body, the centrosome or 'centriole', is an extranuclear structure developed in a kinoplasmic cap at the position of the future pole of the spindle. Viewed from certain directions it is of the form of a curved rod. It is situated close to the nuclear membrane, and from it radiate delicate kinoplasmic fibres. During these early stages the centrosphere is seen with difficulty, but it becomes more prominent as karyokinesis progresses. A centrosome and centrosphere are seen at either pole of the nucleus during all nuclear divisions, but they are much more conspicuous accompanying the divisions in the tetraspore mother-cell. Immediately below the nucleus and descending into the vacuolated basal region is a prominent inverted cone of cytoplasm, into which, after division, the stalk-cell nucleus will pass. At about this stage the common cuticle, which has enveloped the developing sorus, is ruptured. This rupture is due to the rapid growth of the band of hairs,

and as the tetrasporangial rudiments develop on the proximal side of these hairs and close to them, it is sometimes found that the cuticle over the zone of young tetrasporangia has disappeared prior to their development. During the prophase stages the nucleus is situated immediately above the inverted cone of cytoplasm. The granules on the spireme threads are distinct, though unequal in size, and stain definitely, though not deeply, both with the triple stain and with iron-alum-haematoxylin. In most of the preparations examined there is in the nucleolus a large central vacuole containing distinct fibrillae. This appearance of the nucleolus is similar to that seen in the succeeding division and suggests that material passes out from it to the developing chromosomes.



TEXT-FIG. 2. *a.* The tetrasporangial rudiment just before stalk-cell division, showing vacuolation of the cytoplasm in the basal portion. A spireme has formed in the nucleus; a faint centrosphere is seen at the distal pole, and a very pronounced, inverted cone of cytoplasm appears below the nucleus. *b.* Shortly after stalk-cell division. The cytoplasmic mass containing the smaller, stalk-cell nucleus has not yet separated from the newly formed cross-wall. $\times 560$.

The spireme now becomes segmented, presumably into the chromosomes, which shorten and thicken considerably before being placed on the equatorial plate. The details of this division require no special mention; the spindle is intranuclear, and, as in the meiotic division, the nuclear membrane (already disappeared at the poles) persists at the sides of the nucleus until anaphase. The procedure is similar to that of an ordinary mitotic division in the thallus, and careful countings of this and other mitoses in the vegetative cells give the sporophytic number, about thirty chromosomes. The longitudinal split which functions to separate the half-chromosomes at anaphase is sometimes seen during the prophase stages.

At telophase (Pl. VIII, Fig. 4) the chromosomes form a flat plate, that of the future nucleus of the tetraspore mother-cell being much larger than that destined to become the nucleus of the stalk-cell. This plate-like form of the telophasic chromatic mass persists until the nucleus is fully formed.

The nucleus of the tetraspore mother-cell now begins to increase in size, and at about this stage the cross-wall is formed separating the tetraspore mother-cell from the stalk-cell (Text-fig. 2, *b*).

THE HETEROTYPE OR FIRST MEIOTIC DIVISION OF THE
TETRASPORE MOTHER-CELL NUCLEUS.

In view of the controversy which exists among cytologists as to the nature of the prophase stages of this division, the writer has directed most attention to the nuclear changes occurring preparatory to the heterotypic metaphase.

In order to avoid, as far as possible, any unnecessary complication the terminology used is that suggested by Miss Digby (4), which is outlined as follows:

- 'Thread.' The longitudinal half of an entire univalent spireme or chromosome.
- 'Filament.' An entire univalent spireme, i. e. the spireme resulting from the parallel association of two half univalent spiremes or threads. (This constitutes the 'leptonema' stage.)
- 'Strands.' Very fine strands of linen (i) connecting the chromosome segments of early telophase; (ii) transversely connecting the individuals of a pair of conjoining or disjoining filaments.
- 'Association.' The coming together, in pairs, side by side, of two threads to form the entire univalent spireme or filament.
- 'Fission.' The longitudinal separation of the univalent spireme into threads or the longitudinal separation of a univalent chromosome into two daughter chromosomes.
- 'Conjunction.' The coming together in pairs of two entire univalent spiremes or filaments to form the bivalent spireme which becomes the bivalent or heterotype chromosome. (This is the stage now referred to by cytologists as 'synapsis'.)
- 'Disjunction.' (i) The separation of the bivalent spireme into two entire univalent spiremes, or (ii) the separation of the bivalent or heterotype chromosome into two entire univalent chromosomes.

It is not easy to find really good examples of the nucleus at rest between stalk-cell division and the early stages of meiosis. This is because the interval between telophase of stalk-cell division and the onset of the prophasic stages of meiosis is very short. The nucleolus takes the chromatin stains very deeply, and the remainder of the nuclear cavity is occupied by a fine, regular, faintly staining reticulum. Only where the threads cross one another are granular structures seen, and these stain deeply with Heidenhain's iron-alum-haematoxylin. At a slightly later stage diamond-shaped meshes are seen and the nucleus is in very early prophase. These spaces or meshes may be due to the separation of the 'threads' (half a univalent chromosome) of the preceding telophase. Careful focusing suggests that this mesh is of the nature of a thin fenestrated sphere with the nucleolus lying in a more or less central position (Pl. VIII, Fig. 5).

As in the stalk-cell division the first indication of approaching mitosis is the aggregation of the reticulum into a very thin spireme—the leptotene stage. It is almost impossible at this stage to tell whether the spireme is continuous or not. As development proceeds the spireme stains more deeply with the basic dyes, and a vacuole appears in the nucleolus. At this stage a smaller body, with a slightly less regular outline than that of the nucleolus, makes its appearance and persists until chromosome formation. This structure is not mentioned in the papers by Georgevitch (6). A similar body has been described in *Dictyota* (18) and styled the 'chromophilous spherule'. This terminology will be retained for the corresponding body in *Padina*. As in *Dictyota* this spherule is only seen during heterotypic prophase. It is possible that this body is of the nature of a chromatin nucleolus or karyosome, i.e. it is chromatin which has been elaborated by the nucleus during the brief resting stage and collected into this small mass from the resting reticulum. It is undoubtedly different in composition from the much larger true nucleolus, as is evinced by its staining reactions with certain dyes such as brazilin or safranin. In sections treated with iron-alum-haematoxylin this difference is not so marked. Whilst the true nucleolus stains with acid dyes, the 'spherule' takes the basic dyes and is probably composed of basichromatin.

A very careful study was made of this early spireme stage, but no indication of a lateral pairing of leptotene threads (parasynapsis) to give the zygonema stage was seen. This suggests that *Padina* does not conform to the mode of reduction explained by Grégoire (8), Berghs (1), and others, and outlined by Sharp (17) as 'Scheme A'.

The nucleus now enlarges slightly, a phenomenon described by Sharp, Lawson (10), and others, and accompanying this increase in the size of the nucleus there is a separation of the spireme from the nuclear wall (Pl. VIII, Fig. 6). This constitutes the 'first contraction' stage preparatory to synizesis.

SYNIZESIS.

The long, thin, and deeply staining spireme which has been contracting away from the nuclear wall now passes into synizesis, in which the spireme thread becomes densely coiled up, generally towards one side of the nucleus. In the majority of the numerous examples of this stage which have been examined by me, this knot appeared towards the proximal side of the nucleus, i.e. the side near the stalk-cell. In a few areas on some thalli, all the synaptic knots were disposed at the distal side of the nucleus, as mentioned by Georgevitch (6); and in a few exceptional cases they were concentrated into two masses at opposite sides of the nucleus and joined by one or two connecting strands, as recorded for *Dictyota* (18). At this stage the centrosphere is not visible, but the knot or knots are disposed at

what are destined to be the future poles of the nucleus. In some cases the knot is in such close contact with the nuclear membrane in this region that it almost suggests actual continuity with the cytoplasm outside. Sometimes the thread is eccentrically coiled into a tangled mass (Pl. VIII, Fig. 7); in other cases loops arise from the skein and project into the nuclear cavity (Pl. VIII, Fig. 8), or, frequently, a few loose ends project irregularly from the knot. The nucleolus is usually in close contact with this chromatic knot, and in some cases becomes pear-shaped, the apex of the conical form being directed towards the mass with some portions of the spireme attached to it. At this stage vacuoles appear in the nucleolus, though not invariably three as recorded by Georgevitch (6), and it appears probable that some of its contents pass out and are used, in some way or other, by the spireme filaments. The remainder of the nuclear cavity is now occupied by a faintly staining reticulum of nucleoplasm which is more prominent during synizesis, and is probably thrown down from the enchylema, as Wilson (19) and others have suggested. Situated in this reticulum, and quite apart from the chromatic knot, is the 'chromophilous spherule'. Whatever the nature and function of this structure may be, it is certainly not, during this stage, in intimate contact with the chromatic mass. No useful purpose would be served here by detailing the various views which have been held as to the possible interpretations to be ascribed to this interesting stage. It seems quite certain from this investigation that, whilst the contraction takes place quickly, synizesis itself persists for some time. It is also evident that the spireme during and after synizesis (pachynema) is much thicker than before this stage, hence there is no difficulty in identifying pre- and post-synizesis phases in prepared slides. From a close study of this and later stages, the most plausible explanation seems to be that during synizesis the two halves (threads) of the univalent spireme (which had separated at the preceding telophase) become associated together to form the univalent spireme (filament). This association of 'threads' is very different from conjunction (the pairing of entire univalent spiremes), which certain cytologists maintain takes place during this stage of synizesis. Lawson (10) suggested that no contraction takes place, and that the characteristic appearance is brought about and can be explained by the enlargement of the nucleus which takes place during this stage. This hypothesis, which has been discounted by Farmer (5) and others, does not hold in the case of *Padina*.

'HOLLOW SPIREME' TO THE 'RESTING' STAGE.

As the nucleus passes out of synizesis, loops of free spireme loosen out into the nuclear cavity, until the whole space becomes filled with the chromatic filament. Separation of this filament into its constituent threads is usually evident at this stage, though the extent to which this separation can be seen varies considerably. In some nuclei it is very conspicuous

(Pl. VIII, Fig. 9), whilst in others the spireme appears as a row of fluffy beads. The nucleolus by this stage has resumed its normal spherical shape, and it generally shows a single large vacuole. The filaments continue to shorten, thicken, and take the chromatic stains more readily. The spireme is disposed towards the periphery of the nucleus, and is sometimes actually in contact with the nuclear wall. A similar phenomenon has been observed and described by Farmer and Moore (5). At a slightly later stage the spireme is more evenly distributed throughout the nuclear cavity, and 'dissociation' (the separation of the threads) is often very well marked, the granules on the threads being more widely separated from each other, and taking the basic stains more readily.

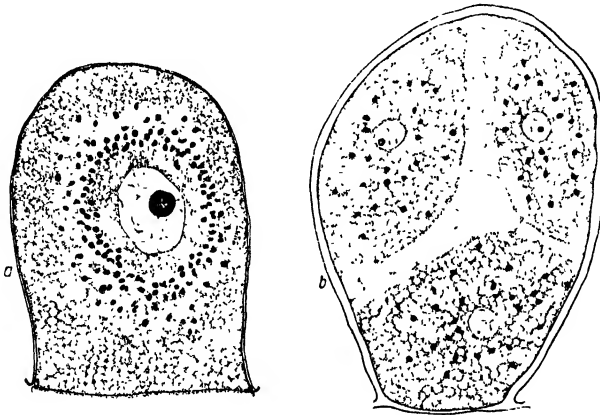
THE 'RESTING' OR 'GROWTH' STAGE.

The next stage in the developing tetraspore mother-cell is interesting, though by no means easy to interpret. Just at the stage in the developing nucleus when one would expect to find a form of 'second contraction' taking place, a peculiar condition intervenes. This is the stage which has been fully described for *Dictyota* as the 'resting stage' (18). From the examination of a number of plants the writer is satisfied that this peculiar phase is quite normal in the history of the developing tetraspore mother-cell, and is not due to an unhealthy condition of the plants examined. The filaments do not show the more or less interlaced and twisted form of the strepsitene condition, but instead appear to separate and fill the nuclear cavity with a number of faintly staining threads (Pl. VIII, Fig. 10). It seems possible, as suggested for *Dictyota* (18), that the threads do separate widely, and it is certainly almost impossible to identify the constituent chromatic elements. The account given for *Dictyota* is the only record I have been able to trace of this phenomenon occurring in plants, and it seems probable that it is peculiar to members of this family. Georgevitch (6) does not mention this phenomenon in his papers. In the case of the animal egg, however, there is an analogous condition which has been described as the 'growth' stage. Perhaps it will not be out of place to quote here the description given by Sharp (17, p. 233) of this growth phenomenon in animal tissues:

'During the relatively enormous growth of the oocyte . . . the chromosomes, which have usually reached the strepsinema stage when the enlargement begins, become greatly modified in form. Their achromatic framework takes the form of fine threads extending out in all directions, giving the chromosomes an irregular brush-like form, while the chromatic substance either may flow into the nucleolus, leaving the chromosome framework uncoloured and very difficult to observe, or by loss of its staining capacity through chemical change it may disappear from view completely. As the growth period comes to an end, however, the original staining capacity

returns, and the chromosomes again assume the compact form and pass into the diakinesis stage.'

It is possible that some similar interpretation might apply in this process in *Padina*. Whilst this stage is in progress there is some enlargement of the tetraspore mother-cell, and the cytoplasmic contents present a characteristic feature. Instead of the plastids being distributed fairly evenly throughout the cytoplasmic reticulum, they are aggregated together into a zone surrounding the nucleus, but separated from it by a narrow zone of kinoplasm (Text-fig. 3, *a*).



TEXT-FIG. 3. *a*. The 'resting' or 'growth' stage. The much-enlarged tetrasporangium, showing the very characteristic arrangement of the plastids. They are aggregated into a zone surrounding the nucleus and separated from the nuclear membrane by a narrow zone of kinoplasm. *b*. The delimitation of the tetraspores. Vacuoles appearing in the zones of clear cytoplasm between the four tetraspore rudiments. $\times 560$.

CONJUNCTION.

As the resting stage passes, the chromatic elements of the nucleus are seen more clearly. The 'threads' which had appeared to separate during the rather unique preceding phase now approximate together again, and the spireme filaments, which take the chromatin stains more readily, proceed to conjoin with each other. Parts of them are now pulled into parallel positions, and this tendency of the sides of the loops to incline towards one another constitutes the first sign of conjunction and the first step in the bringing together of the univalent chromosomes to form the characteristic heterotype bivalents. Some filaments merely run parallel with each other and are joined at intervals by fine strands; others are twisted over one another, and the ends of the filaments may or may not diverge (Pl. VIII, Fig. 11). Fission of the univalent elements is not generally evident, and can only be seen in the free ends of some conjoining loops (Pl. VIII, Fig. 12). Very occasionally two conjoining pairs lie near each other and fine, though definite, strands connect them. It is difficult to offer an interpretation of

this phenomenon. The writer did not find a nucleus in which all the spireme filaments showed definite conjunction at the same time. In some preparations many examples were seen in a single nucleus, in others only one or two pairs of filaments were found showing this formation of bivalents. The centrosome and centrosphere become visible about this stage, and the nuclear cavity, which has been increasing in size since the very early prophase, now attains its largest dimensions.

DIAKINESIS.

The double structures shorten and thicken until finally, in the diakinetik stage, the bivalent chromosomes are formed. These stain like homogeneous bodies. In one or two preparations examined these bivalent chromosomes (gemini) were disposed at the periphery of the nucleus, as though separated by mutual repulsion. There seems to be a variety of ways in which the univalent chromosomes in each bivalent combination may arrange themselves with reference to one another. Some take the form of paired rods ||, others of crosses X, whilst the ring form O is very frequent. Both Lloyd Williams (18) and Georgevitch (6) have remarked on the prevalence of the closed ring form of bivalents in this type. Only rarely can fission of the constituent univalent chromosomes be detected at this stage. This fission is a reappearance of that split which was so evident in the post-synizesis filaments, and which will function eventually in the separating of the daughter univalent chromosomes at the homotypic division of meiosis. There is a slight decrease in the size of the nucleus during diakinesis, and the nuclear membrane is usually very conspicuous. The nucleolus shows many vacuoles containing numerous fibrillae. Much has been written by Moore (12) and others on the changes in the nucleolus during mitoses and its possible function in the nucleus. From its intimate contact with the spireme during synizesis, and its vacuolated appearance during other prophase stages, it seems probable that it is a reservoir of material which is used up in the development of the chromatin filaments. It may have other functions as well, and possibly it has different functions in different cells. Shortly after the formation of the bivalents the spindle fibres begin to appear. It has not been possible to decide definitely how these originate. At about this stage, or sometimes a little earlier, the nuclear membrane shows a pronounced depression at the poles, due probably in part to the action of the enlarging centrospheric radiations. It is significant, too, that the nuclear membrane gradually disappears at the poles prior to metaphase, though elsewhere it persists, practically unchanged, until anaphase. Careful examination, however, suggests that the spindle fibres are formed from nuclear matter *in situ*, and are not cytoplasmic radiations which have penetrated the nuclear cavity from the region of the centrosphere.

Very careful counts of the bivalents at diakinesis have been made in

many nuclei, and the number appears to be sixteen. There are, therefore, thirty-two univalent chromosomes, with sixteen as the reduced number. Georgevitch (6), however, states that the sporophytic number of chromosomes is twenty-four, with twelve as the number in the gametophytic generation.

THE HETEROTYPIC SPINDLE.

After formation of the gemini, shortening and thickening proceeds until metaphase, when the bivalents are definitely homogeneous in appearance, compact, and deep-staining. The nuclear cavity is now much smaller. The chromosomes are now arranged on the equatorial plate of the bipolar spindle, and here again the number of bivalent chromosomes is apparently sixteen. At this stage they are small and usually more or less oval in shape, and owing to their size it is not easy to decide how they are attached, but it appears that they are fastened so that both univalent chromosomes are in contact with the spindle fibres. As has been described in other cases, the spindle fibres which extend uninterruptedly from pole to pole are usually taut. Other fibres extend from the pole to the chromosomes; these are the 'half-spindle' or traction fibres. Still other lax fibres radiate from the poles and end in the nucleoplasm between the spindle and the nuclear membrane. During metaphase stages in particularly good preparations, one sees very minute fragments of stained matter strewn along the spindle fibres at the region of the equatorial plate, an appearance suggesting the adhesion of stained particles to the fibres. The curved centrosome is usually very evident, but the centrosphere is not so pronounced as it is either during conjunction, or, later, at telophase.

In all the preparations showing this stage, the axis of the spindle is either at right angles to the surface of the thallus or slightly oblique. This plane of division is no doubt effected by the lateral pressure resulting from the crowding of the sporangia into zones, thus producing the flask-shaped or globular tetrasporangia. Hertwig (9) and others (19) point out that the usual position of the nucleus, and hence of the mitotic figure, tends towards the centre of its sphere of influence, and that the axis of the spindle typically lies in the longest axis of the protoplasmic mass. The reaction of the spindle towards general cytoplasmic shrinkage is of interest. In well-fixed preparations there is present an appreciable nuclear space between the equatorial plate and the nuclear wall. Examination of material in which shrinkage has taken place shows that the nuclear membrane also has contracted on to the spindle, which, however, retains its normal size, apparently resisting the external pressure (Pl. VIII, Fig. 17).

Disjunction of entire univalent chromosomes takes place at early anaphase, and the nuclear membrane, which has persisted excepting at the poles until this stage, now disappears. It is sometimes found that three of the chromosomes lag behind the others on their way to the pole and

project towards the equator. Many cytologists have observed that certain chromosomes are recognizable in the chromosome group at each mitosis. Characteristic sizes, shapes, and regularly situated constrictions assist in this identification. During anaphase, in certain univalent chromosomes, the split which will function to separate the two halves of this unit at the metaphase of the next division is well marked. This splitting does not proceed throughout the entire length of the chromosome. Sometimes also delicate strands are seen which join up the chromosomes laterally (Pl. IX, Fig. 19), and which are evidently a preparation for the succeeding telophase.

The chromosomes join up together into two or more darkly staining masses at late telophase, but never actually abut on the centrosome; they never approach nearer to the centrosome than is shown (Pl. IX, Fig. 20). The telophasic group is capped by prominent kinoplasmic fibres, suggesting very strongly that these fibres assist in the formation of the nuclear membrane. The spindle fibres gradually disappear as the daughter nuclei are reconstructing. Vacuolation of the chromosomes proceeds and their outline is soon lost to view, whilst a definite nucleolus forms in both nuclei. The homotype division in this plant, therefore, does not follow the heterotype division as quickly as is the case in some other plants. A distinct reticulum is formed in the nucleus, and the chromoplasts, which have been multiplying during this nuclear division, are generally distributed uniformly around the two daughter nuclei, leaving the median zone clear. This corresponds to the condition described for *Dictyota* (18).

THE HOMOTYPIC OR SECOND MEIOTIC DIVISION.

The prophase stages of this division are passed through rather quickly. The nucleus enlarges and is seen to contain a delicate linin reticulum, in which are scattered the small chromosomes, and a large nucleolus with a vacuole containing fibrillae. At the time when the spindle fibres begin to enter the nuclear cavity (Pl. IX, Fig. 23), the centrosomes with their kinoplasmic caps are at an angle apart of about 140° ; so the spindle cones begin to appear before the centrosomes have reached the future poles of the nucleus. This is different from the procedure in the previous heterotypic division, where the centrosomes have traversed to opposite sides of the nucleus before the spindle fibres enter the nuclear cavity. As in the preceding division, lax mantle fibres also radiate from the poles into the nucleoplasm. The axes of these homotype spindles are at right angles to that of the preceding heterotypic spindle, i.e. they lie in a plane parallel to the surface of the thallus. The two homotype spindles may lie parallel to one another, or at right angles to each other. These spindles are undoubtedly smaller than the heterotypic spindle, and the nuclear cavity occupied by the spindle much narrower. The spindle is often distinctly

'waisted' at metaphase and does not fill the nuclear cavity (Pl. IX, Fig. 24). No example was seen of an extremely narrow, curved spindle as figured in *Dictyota* by Mottier (13) and Lloyd Williams (18).

It will be seen that the chromosomes are distinctly homotypic in character and the centrosphere prominent at either pole. Chromosome counts were made from oblique views of this metaphase and anaphase, and, in spite of the number of small chromosomes, there is no doubt that the number corresponds to that of the bivalent pairs, i. e. that numerical reduction has been accomplished.

The daughter nuclei are formed as in preceding divisions.

FORMATION OF TETRASPORES.

After this second division the plastids are scattered throughout the tetraspore mother-cell. The four nuclei now separate from each other, and this movement of the nuclei is followed by a rearrangement of the plastids, which are disposed around each nucleus as centre, leaving a zone of clear cytoplasm between each group of plastids. Vacuoles now form in this clear cytoplasm; these unite to form furrows, and in this way four free independent tetraspores are formed packed close together within the wall of the mother-cell (Text-fig. 3 *b*). A typical tetraspore shows a narrow zone of kinoplasm surrounding the nucleus and a distinct radiate arrangement of the plastids. The four tetraspores within the wall of the mother-cell are not, at first, enclosed within definite membranes, and they enlarge considerably before being liberated.

THE GERMINATING TETRASPORES.

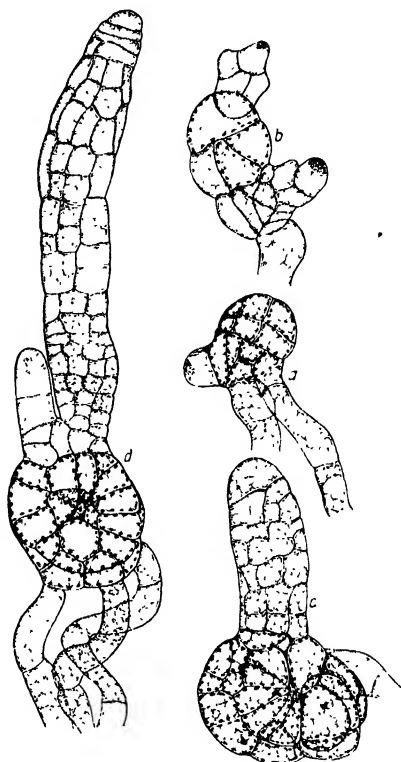
The early development of the germinating tetraspore has been briefly described by Reinke (15), and the stages examined by the writer do not differ materially from those figured by this earlier worker.

Before actual division the tetraspore enlarges considerably until it almost equals the size of the mature tetrasporangium. After the first division into two cells and a further division in each of these at right angles to the plane of the first division, many divisions take place in different planes without, apparently, following any fixed sequence. The result of the divisions is the production of a multicellular, more or less oval structure which forms the so-called 'central nodule' of the new *Padina* plant. This 'nodule' is easily recognized by the much darker colour of its cell-contents. Concurrently with these divisions one or more of the superficial cells give rise to filamentous rhizoids which at first are unbranched. These serve to fix the young plant to the substratum. One of the superficial cells of this central nodule then becomes the mother-cell of the young *Padina* plant. Often this mother-cell lies in the longitudinal axis of this central nodule, but sometimes it occupies another position (Text-fig. 4, *a*). This mother-cell

arises first as a protrusion; a cross-wall then divides this into an apical cell and a basal portion. Additional segments are cut off from the apical cell, whilst in the older parts longitudinal divisions occur. This new growth arising from the central nodule is the 'Rundtrieb'. Further cell multiplication takes place, and, at about the stage shown (Text-fig. 4, *d*), this 'Rundtrieb', which is circular in section, gives rise to the 'Flachtrieb'. In this latter stage the longitudinal of the segments which are cut off occur almost entirely in one plane. Consequently, whereas in the 'Rundtrieb' the cell tissue is arranged radially around an axis, in this 'Flachtrieb' the cells are more or less parallel to each other. Later, apparently from this 'Flachtrieb' the fan-shaped 'Breittrieb' arises. Other superficial cells of the same central nodule can likewise grow out into 'Rundtriebe' (Text-fig. 4, *b*). Meanwhile, the rhizoids have lengthened and become branched. As this paper is concerned mainly with the cytology of the sporophyte generation, that of the germ-lings and the developing sexual cells will be dealt with in a subsequent paper.

ABNORMALITIES.

1. *Twin tetrasporangia.* These are of fairly frequent occurrence. Stalk-cell division is quite normal, and there is no difference in size between these rudiments which give rise to twin tetrasporangia and those which produce normal ones. Instead of the nucleus passing into the normal spore prior to synizesis, it divides in a somatic manner with the axis of the spindle parallel to the surface of the thallus. Two daughter-cells are thus formed which lie side by side over a single stalk-cell. These twins then pass through the usual heterotypic divisions, but, as recorded by Lloyd Williams (18), the nuclear changes in them occur somewhat later than in normal contiguous tetrasporangia.



TEXT-FIG. 4. Portions of germinating tetraspores. *a* The 'central nodule' with the 'Rundtrieb' beginning to develop. The bases of two rhizoids are seen. *b*. Two 'Rundtriebe' developing from one central nodule. *c*. A many-celled 'Rundtrieb', circular in section, produced by rapid division. *d*. A much later stage of development; at about this stage the cylindrical 'Rundtrieb' gives rise to the flattened 'Flachtriebe'.
× 15c.

2. In one specimen examined tetrasporangia were developed on both sides of the thallus. The development in this case was quite normal.

3. A few cases were observed in which a tetrasporangium rudiment had cut off two stalk-cells; both equal in size, instead of one. Here also the later development was normal.

SUMMARY.

1. A detailed study of the mode of growth of the fan-shaped thallus confirms the conclusion arrived at by Reinke that it takes place by division of a row of marginal cells. By differential growth of the segments cut off the margin of the thallus is rolled inwards.

2. The concentric zones of hairs originate near the apex as outgrowths of special superficial cells, and are not outgrowths of entire surface cells, as is suggested in some published figures. These hair rudiments are cut off by division from surface cells, and in section appear triangular. By rapid basal division they soon attain a considerable length. The reproductive cell rudiments arise on the proximal side of these bands of hairs.

3. The tetrasporangia also originate in concentric zones, and all those in the same zone are generally at about the same stage of development.

4. The sequence of events in the early development of the tetrasporangia (viz. the enlargement of the tetrasporangial rudiment, the separation of the stalk-cell, and the stages preparatory to synizesis) is very similar to that obtaining in *Dictyota*. Many of the nuclear changes are passed through very quickly, and the interval between stalk-cell division and the onset of the meiotic division is short.

5. The univalent spireme results from a close approximation of two 'threads', i.e. the halves of the univalent chromosomes which separated at the preceding telophase, and this association takes place during synizesis. The vacuolation of the nucleolus and its intimate contact with the developing spireme suggests that material passes from it during this stage. A 'chromophilous spherule' is also present and persists until diakinesis.

6. As in *Dictyota* a characteristic 'resting' or 'growth' stage is interpolated between 'open spireme' and 'strepsinema'. During this stage the chromatin takes the basic stains very feebly; the chromosomes become vacuolated and their identity is lost. Meanwhile the chromoplasts are arranged in a zone surrounding the nucleus, but separated from it by a narrow zone of kinoplasm.

7. The bivalent chromosomes are formed as the result of the conjunction of two univalent filaments initiated by the looping of the spireme threads and the production of strands to draw the parallel filaments together. This conforms, in essential features, to the mode of formation of bivalents described by Farmer and others and outlined by Sharp as Scheme 'B'.

8. The number of bivalents at diakinesis is apparently sixteen, i.e. the

number of chromosomes characteristic of the sporophyte generation is thirty-two. The ring form **O** of arrangement of univalents to form gemini at this stage is very frequent.

9. The spindle of the first meiotic division is almost invariably at right angles to the surface of the thallus and is intranuclear. A curved centrosome and a centrosphere are prominent at either pole. In the first (heterotypic) division of meiosis there is undoubtedly a reduction in the number of chromosomes; entire univalent chromosomes separating at this metaphase. The reduced number, counted at this anaphase and during the succeeding division, is apparently sixteen.

10. In a number of cases the split which is to function during the following homotype division becomes visible during the heterotypic anaphase.

11. During the fairly short interphase between the two meiotic divisions a nucleolus appears in the nucleus, but there is no stage of complete rest. The spindles of the homotype division lie in planes at right angles to that of the heterotypic spindle, and are often distinctly 'waisted'. In this division the already split halves of the univalent chromosomes separate to form the four daughter nuclei.

12. In the four-nucleate condition the plastids are orientated around the nuclei, leaving the intervening cytoplasm clear. Vacuoles which appear in these zones of clear cytoplasm join up, thus delimiting the four free tetraspores.

13. Germination of the tetraspore, as outlined by Reinke, takes place after a short rest. An oval mass of cells (the 'central nodule') gives rise to filamentous rhizoids and to cylindrical 'Rundtriebe'; This later gives rise to the more flattened growth, the 'Flachtrieb', and apparently later to the fan-shaped 'Breittriebe'.

In conclusion I wish to express my thanks to Professor Lloyd Williams for suggesting this research and for much advice and criticism; also to my colleague, Dr. S. G. Jones, for his assistance during the progress of the work. I am also indebted to the Director of the Marine Biological Station, Naples, and to Professor Funk for selecting and forwarding material.

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EXPLANATION OF PLATES VIII AND IX.

Illustrating Mr. P. W. Carter's paper on *Padina Pavonia*.

All figures have been drawn with the aid of a camera lucida immersion lens, Leitz $\frac{1}{2}$ " apochromatic with ocular 20. \times 2,800.

PLATE VIII.

Figs. 1-3 and 5-21 have the stalk-cell on the lower side, and the terms 'proximal' and 'distal' are used with reference to this stalk-cell.

Figs. 1-4. Stalk-cell division.

Figs. 5-22. First meiotic division.

Figs. 23-6. Second meiotic division.

Fig. 1. Prophase of stalk-cell division. The rather coarse spireme is beginning to segment. A centrosome and centrosphere are seen towards the distal side.

Fig. 2. Later prophase, showing a number of small chromosomes and the nucleolus fragmenting. A centrosphere is seen at either pole, and spindle fibres entering the nuclear cavity from the distal pole. The section is not a median one.

Fig. 3. Metaphase. A slightly oblique section with some of the chromosomes. The spindle is intranuclear.

Fig. 4. Telophase. The chromosomes form a flat plate. The tetraspore mother-cell nucleus (the lower one) is larger than that of the stalk-cell.

Fig. 5. Very early prophase of the first meiotic (heterotypic) division. The linin reticulum presents the form of a diamond-shaped mesh distributed over the surface of the nucleus. A faint vacuole appears in the nucleolus.

Fig. 6. Enlargement of the nuclear cavity and withdrawal of the spireme from the periphery. This stage represents that of 'first contraction'.

Fig. 7. Synizesis. The spireme contracted into a knot at the proximal side of the nuclear cavity. The nucleolus drawn out into a pear-shaped process and in intimate contact with the spireme. The 'chromophilous spherule' is situated in the nucleoplasmic reticulum and apart from the chromatic knot.

Fig. 8. A slightly later stage. The spireme is thrown into very pronounced loops with a free end thrown out towards the right.

Fig. 9. Open spireme. The filaments are definitely double, much thicker and deeper staining than the pre-synizesis spireme. At *a* a looped portion is figured.

Fig. 10. 'Resting' or 'growth' stage. Characterized by the very faint staining and the almost complete loss of the identity of the chromatin elements. The 'spherule' is seen near the nucleolus.

Fig. 11. Early conjunction. Some of the filaments show a tendency to fall over in loops. Fission is visible in some of the filaments.

Fig. 12. A slightly later stage, showing looping over and conjunction of univalents forming figures typical of the heterotypic bivalents. The 'spherule' is seen towards the proximal side of the nuclear cavity.

Fig. 13. Shows the conjunction of univalents, i. e. filaments. Note that the sides are drawn in towards one another and connected by fine transverse strands.

Figs. 14, 15. Diakinesis, presenting the different arrangements of univalents in the bivalent pairs. The 'ring' form is evident. At *a* fission is seen in a univalent chromosome.

Fig. 16. Metaphase of the first meiotic division. The chromosomes, still more concentrated and distinctly heterotypic in character, are arranged on the equatorial plate. The spindle is intranuclear and lax mantle fibres also radiate from the poles. The nuclear membrane is still visible at the sides.

Fig. 17. Shows a metaphase from a tetraspore mother-cell in which shrinkage has taken place. The spindle has resisted the external pressure.

PLATE IX.

Fig. 18. Early anaphase. The spindle and 'half-spindle' fibres are very clear.

Fig. 19. Late anaphase. The split which will function at the succeeding homotype division is evident in several of the univalent chromosomes towards the distal pole. Strands connecting the chromosomes are also seen at *a*. Some chromosomes lag behind the others, pointing towards the equator.

Fig. 20. Telophase. The chromatin masses never approach nearer the centrosome. The connecting spindle fibres have nearly disappeared. The polar radiations have become more pronounced.

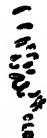
Figs. 21, 22. Two daughter nuclei. Many of the chromatin masses appear double.

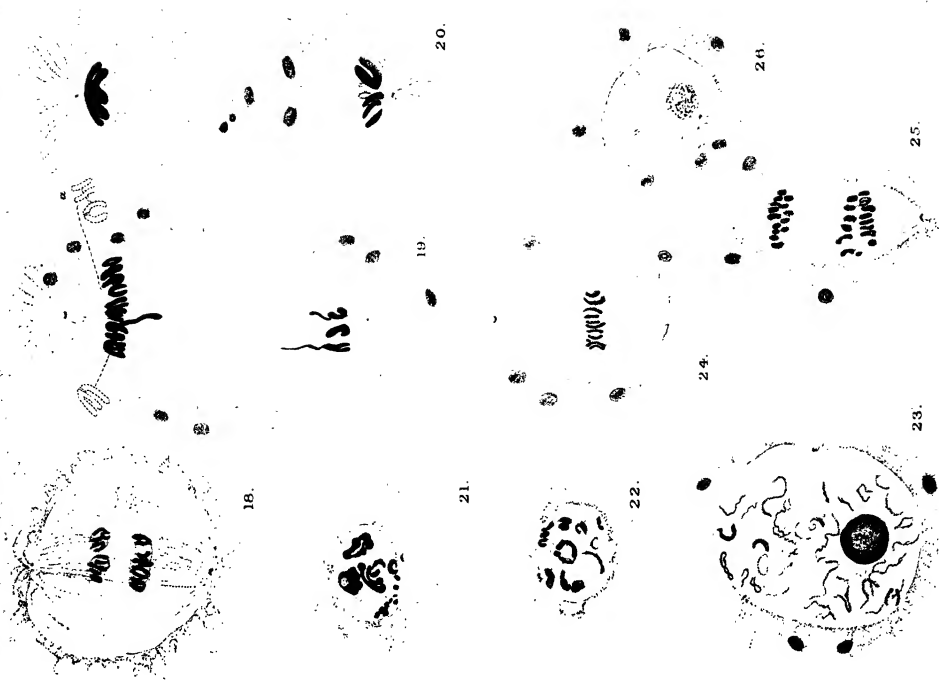
Fig. 23. Prophase of the second meiotic (homotype) division. The nucleus has enlarged, and chromosomes can be identified in the nuclear cavity. The nucleolus contains fibrillae. Spindle fibres begin to appear, although the centrosomes do not yet lie at opposite sides of the nucleus.

Fig. 24. Metaphase of this division. The spindle is narrow and distinctly 'waisted'; the nuclear membrane still intact except at the poles.

Fig. 25. Anaphase. An oblique section showing most of the chromosomes.

Fig. 26. One of the four tetraspore nuclei before rupture of the wall of the tetraspore mother-cell.





RWC del.

CARTER — PADINA.

Hutch. lib. et. imp.

Crinalium, a New Genus of Cyanophyceae, and its Bearing on the Morphology of the Group.

BY

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With two Figures in the Text.

WHILST examining a collection of fresh-water algae, made by the writer during the summer of 1921 in various localities in North Wales, specimens of a blue-green alga, apparently of a new type, were encountered. A description of the latter alone is given in this paper, since the majority of the algae found in the same collection show no peculiarities of structure or distribution worthy of record, whilst the type in question, although closely allied to known forms, shows morphological features of some importance.

The material in which these new organisms were found consisted of a blue-green jelly scraped from the damp face of a rock in Fairy Glen, Bettws-y-Coed, North Wales, collected by the writer on August 22, 1921, and preserved in 4 per cent. formalin. The bulk of this material proved to consist of the gelatinous colonies of *Aphanocapsa fonticola*, Hansg., and the adult organisms in question are found endophytic in the mucilage of this *Aphanocapsa*. The specimens consist of filaments of typical Cyanophycean character, embedded in the mucilage of the host. Thus they are blue-green in colour and differentiated into trichome and sheath, absorb protoplasmic stains, and, with iodine, show the presence of glycogen. They are undoubtedly members of the Cyanophyceae. The organisms resemble in habitat the endophytic species of *Lyngbya*, the specific name *endophyticum* being thereby suggested. With the genus *Lyngbya* the new species also agrees in having simple filaments without spores or heterocysts, the latter fact showing that it belongs to the Oscillatoriaceae. Each filament consists in the adult state of trichome and sheath, the latter being thin but clearly defined, membranous, slightly brownish. The plants, however, differ from

those of the genus *Lyngbya* in three essential points, viz. (i) the filaments are doubled by means of a bend about their middle, whilst the two limbs so produced are often coiled in a lax spiral, so that the whole structure has the form of a hairpin; (ii) the filaments are flattened, strap-shaped, as described for *Spirulina duplex*; and (iii) there are no transverse septa visible in the unstained state. From all species of *Spirulina* the filaments differ in the possession of a definite sheath. The facts show that the plants undoubtedly belong to a distinct and hitherto unknown genus.

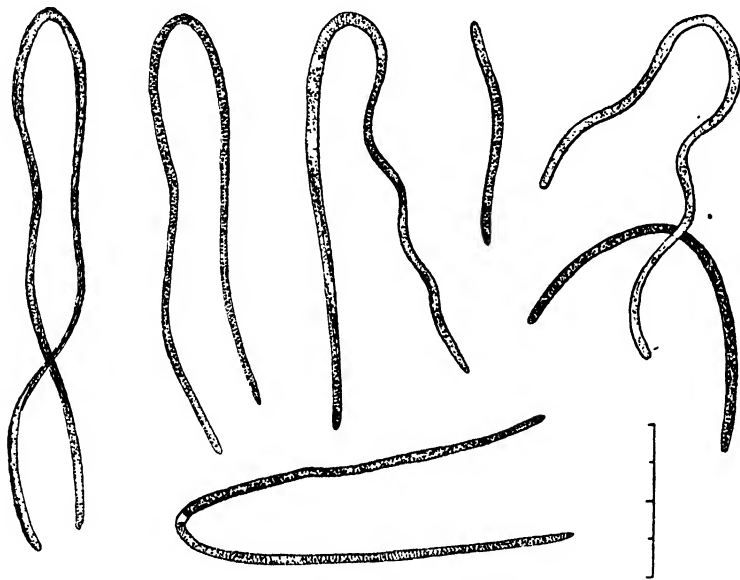


FIG. 1. *Crinalium endophyticum*, n. gen. et sp. Filaments at various stages of development. The scale is $40\ \mu$ in total length.

The filaments are 2 to $3\ \mu$ in width, rarely $4\ \mu$, the thickness of the sheath being less than $1\ \mu$. The end of the trichome is slightly pointed when mature, as shown in the figure. There is no trace of a calyptra or capitate ending as often occurs in this family.

The remarkable form of the filaments has suggested the generic name *Crinalium* (Lat. *crinale*, a hairpin). It is true that not all the adult filaments show this typical form, and it is absent in the young state (hormogone). Yet very few of the adult filaments are without a pronounced bend in the middle. The spiral bend of the filaments is also characteristic although not invariably present. This is quite in harmony with what has been found in studying the variation of other Cyanophyceae, even the most characteristic features, e.g. the spiral form of *Spirulina*, not being absolutely constant. The spiral form is of functional interest in *Crinalium endophyticum*, since it may aid the plant in boring into the host mucilage, just as in *Spirulina* it may function in facilitating swimming in water. The

doubled form of the adult filament in *Crinalium* is apparently antagonistic to a free swimming habit, but the hormogones, which were frequently found outside the mucilage, are sometimes spiral; i.e. when they reach their maximum length. The loose nature of the spiral recalls that of *Spirocoleus*, Moeb. The validity of this genus is being upheld in another publication. *Crinalium* also agrees with *Spirocoleus* in being less fixed in spiral form than is *Spirulina*, Menegh. The hormogones mostly begin to bend when they reach a length of between 100–200 μ . At the same time traces of a sheath become visible. Many of the hormogones were found free from the mucilage of the host, from which they had probably escaped in the active state. They doubtless serve as a means of distribution as in related species. It is possible that the adult filaments show changes of shape in the living condition, but in view of their sheath and embedded habitat this would not appear likely. The regular bend, but not the spiral character, distinguishes this species from all endophytic species of *Lyngbya*, the spiral form appearing in *L. mucicola*, Lemm., in which there are numerous irregular bends, so that this species is allied to *Crinalium*. *Lyngbya Scotti*, Fritsch, is also somewhat similar, but here the greatly contorted threads are united to form a false thallus, whilst the plant is epiphytic rather than endophytic. *L. rivulariarum*, Gom., is also allied, but in the character of the foldings of the filament appears to be even more irregular.

The shape of the filaments of *Crinalium* is of great interest in connexion with the development of the colonies of the Rivulariaceae. The filaments of the youngest colonies of the latter are aggregated side by side, as in the genus *Aphanizomenon*. Later they fold in half so that each adult filament of the colony is really only half a filament that has undergone bipolar differentiation. This is obviously parallel to the development of the always isolated threads of *Crinalium* and affords an instance of a continuation of this developmental tendency in more differentiated forms.

Another significant fact is that it strengthens the belief in an affinity of the Oscillatoriaceae with the Spirochaetes. The similarity of the latter with *Spirulina* has often been remarked, but the difficulty has always existed that species of the latter (excluding *Glaucoospira*, which has been claimed for the Spirochaetaceae) have not the capability of folding in the characteristic manner of a Spirochaete, which always appears to fold into two during the process of division. In *Crinalium* one meets with an undoubted member of the Oscillatoriaceae with a sheath present in which there is no evidence of the extreme mobility of the Spirochaetaceae, yet in which the doubling of the filament occurs, whilst the trichome not infrequently constricts at the angle of the bend, forming two long hormogones.

The characters of *Crinalium* are not unlike those of *Spirulina duplex*, Wolle, particularly in the form of the filament, both as regards its double nature, its spiral form, and its flat shape. The resemblance of *Crinalium*

endophyticum and *Spirulina duplex* is particularly noticeable when, as occasionally occurs, the two limbs of the filament are twined round one another. Setchell and Gardner¹ figure a specimen of *Arthrospira breviar-ticulata* which is not only doubled, but also with the two limbs twisted on themselves in this manner, but apparently this is an abnormal specimen. The specimen is very much bigger than either of the plants in question. Bending of all kinds is of course met with in Oscillatoriaceae. The size of *S. duplex*, Wolle, and *Crinalium endophyticum* is not dissimilar. Those of *S. duplex* are described as $2\ \mu$ in diameter. The length of the double filament of *Crinalium* is also frequently within the limits $75\text{--}200\ \mu$ given for

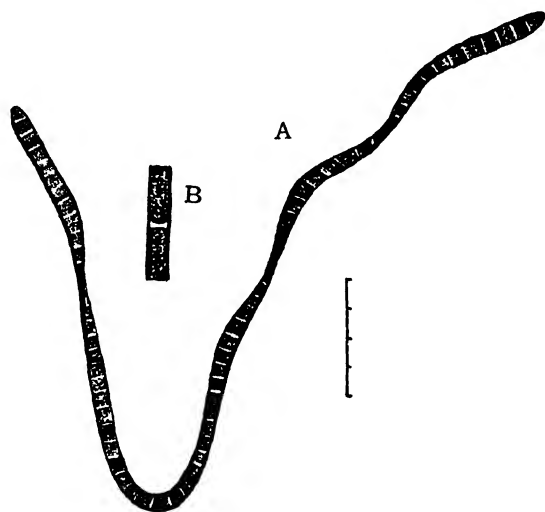


FIG. 2. *Crinalium endophyticum*, n. gen. et sp. A, Filament free from host and stained with neutral red. B, Portion of filament with trichome broken, showing sheath. The scale is $20\ \mu$ in total length.

S. duplex. Also the filaments vary greatly, not uncommonly being more than $200\ \mu$ in length. There are commonly less than four turns in each plant, but in both sometimes more.

The objective existence of *Spirulina duplex* does not appear to have been established since it was described. Wolle's figures illustrate what may have been thickening rings from disintegrating wood elements.

Specimens of *Crinalium endophyticum* were stained by irrigation with neutral red, and, as in many of the spiral forms of the family, transverse septa appeared as clear lines across the contour of the whole filament. They were $3\ \mu$ apart, and so the segments are approximately square in optical section. As in *Spirulina*, the stained filaments show numbers of granules; in this case numerous small additional granules appeared besides

¹ Univ. Calif. Publ., Bot., viii, 1919.

those seen in unstained plants. Whether transverse walls exist in *S. duplex*, Wolle, is not known.

The following is the generic and specific diagnosis :

Crinalium, nov. gen. Trichomes multiseptate, strap-shaped, duplex, but not forming an annulus, generally loosely spiral, vaginate, sheath thin, apex slightly tapering, calyptra absent. Reproduction by hormogones.

C. endophyticum, nov. sp. Filaments not obviously septate, endophytic, 3-4 μ diameter.

SUMMARY.

Crinalium endophyticum, n. gen. et sp., a new member of the family Oscillatoriaceae, is described. The filaments were found endophytic in colonies of *Aphanocapsa fonticola*, Hansg., on the damp face of a rock in North Wales. A sheath is present, the filaments being distinguished from *Lyngbya* in being doubled and flattened. There is a tendency to spiral coiling, but not of so pronounced a kind as in *Spirulina*. Resemblances to Rivulariaceae and to Spirochaetaceae were also observed.

The Role of Boron in the Growth of Plants.

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With Plates X and XI.

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1. ENVIRONMENTAL CONDITIONS AND THE NEED OF BORON FOR GROWTH.

THE important role of boron in the nutrition of *Vicia Faba* was clearly shown in Warington's earlier work (3, 4), but it remained to be proved whether the beneficial action of the element is a general phenomenon or is confined to particular conditions of growth. Repeated experiments have confirmed the statement that broad beans, grown in nutritive solutions composed of salts spectroscopically free from boron,¹ fail to complete their development, but die in a characteristic manner, the growing-points being the first parts affected, whereas with the addition of traces of boric acid normal growth is made. Plants grown in pure washed sand, supplied with nutrient salts, exhibited exactly the same phenomena, dying at the apices

¹ The salts used throughout the work have been examined spectroscopically by Dr. Judd Lewis and declared free from boron.

unless a modicum of boron was also available (Pl. X, Fig. 1). This shows that the effect is not due to the abnormal liquid medium presented by water cultures, as it is also produced on the solid substratum of sand. The failure to obtain the same result in soil has been attributed to the presence of sufficient boron in the soil to supply the needs of the plant.

In water cultures without boron, broad bean roots become short and stumpy, whereas with boron they are long, with numerous thin laterals. This difference does not appear to be connected in any way with the conditions of aeration obtaining in water culture, as exactly similar plants were obtained by growing beans, with and without boron, in non-aerated solutions and in solutions supplied with extra oxygen by running air through at stated intervals. In both cases the typical stumpy root occurred in the absence of boron, and one is forced to the conclusion that this element is the active factor, and that its function is unaffected by the nature of the substratum or the conditions of aeration at the roots.

Another abnormal feature of water cultures is the absence of *Bacillus radicola*, the formation of root nodules being thus inhibited. Plants grown in solutions inoculated with virile cultures of the organism, with and without boron, behaved in exactly the same way as parallel non-inoculated sets, demonstrating that the absence of nodules plays no part in determining the effect of boron. These cultures led to detailed investigation of nodule structure, which is vitally affected by the absence of boron, as has already been described elsewhere (1). In this connexion it is interesting to note that in sand cultures beans grown with boron developed, from casual infection, a fair number of nodules, varying from 13 to 146 per plant, whereas those without boron produced no nodules except for nine on one plant only (Fig. 2).

In all the preliminary water-culture work on the effect of boron on plant growth, the Rothamsted nutrient solution, pH 3.8 (3, p. 631), was employed. The question arose as to whether the results indicating the essential nature of boron for certain plants were merely due to something inherent in the composition of this solution, or whether similar results would be obtained if the plants, e.g. broad beans, were grown in solutions of (1) practically the same composition, balance, and concentration, but with altered pH value, and (2) entirely different composition, with consequent alteration in balance, concentration, and pH value.

(1) The Rothamsted culture solution was modified by replacing the KH_2PO_4 by such proportions of K_2HPO_4 and KH_2PO_4 as would change the pH value from 3.8 to (a) 5.0 and (b) 6.2, but allow the total phosphate to remain unaltered. A slight increase in the potash content of the modified solutions was unavoidable.

The characteristic dying appeared in all plants which were not supplied with boron, irrespective of the pH value of the nutrient solution. Within

the limits of pH 3.8-6.2, therefore, the degree of acidity of the culture solution in no way interfered with the effect of the omission of boron. However, as is generally found, the pH value of the solutions noticeably affected the growth of the plants, particularly in the early stages, development being the more rapid as the acidity decreased. As a result, although the time elapsing before the withering of the shoots appeared was much the same in all three solutions, plants in the culture of pH 6.2 made much better growth before the symptoms of a deficiency of boron appeared than did those in the more acid solutions. Similarly, where boron was supplied, although all the plants flowered and were normally healthy, those in the least acid solutions made the strongest growth.

Similar experiments with solutions from which the nitrate was omitted, but in which the plants were supplied with a potential source of nitrogen by inoculation with *Bacillus radicola*, fully confirmed the above result.

(2) To determine the relation between the utility of boron and the composition of the nutrient medium, broad beans were grown in the following solutions,¹ both with and without the addition of boric acid :

Solution.	pH Value.
1. Rothamsted pH 6.2 ²	6.2
2. Modification of Rothamsted pH 3.8 ³	below 3.5
3. Detmer ⁴	" 3.5
4. Crone ⁵	6.1
5. Modification of Moore ⁶	7.3
6. Knop (osmotic concentration) 1.75 atm. ⁷	4.5
7. Tottingham T ₃ R ₁ C ₄ (osmotic concentration 1.75 atm.) ⁷	4.5
8. Tottingham T ₁ K ₇ C ₁ " " 2.5 " ⁸	4.6
9. Shive R ₃ C ₂ (osmotic concentration 1.75 atm.) ⁷	4.5
10. Allison and Shive (osmotic concentration 1.75 atm.) ⁹	4.5

All plants supplied with boric acid, with the exception of those in the variant of Moore's solution, were perfectly healthy and flowered, although the amount of growth made varied considerably with the different media (Table I). The modification of Moore's solution proved extremely unfavourable. This was probably due to the presence of an excessive amount of magnesium in proportion to the calcium, and possibly also to the

¹ Spectroscopic examination was not made of the Ca₃(PO₄)₂ and (NH₄)₂SO₄ used in Crone's and Moore's solutions respectively.

² KNO₃ 1 grm.

KH₂PO₄ 0.3 grm.

K₂HPO₄ 0.27 "

MgSO₄ 0.5 "

CaSO₄ 0.5 "

NaCl 0.5 "

Fe₂Cl₆ 0.4 "

Distilled H₂O 1 litre.

⁷ Physiol. Res., i, p. 393.

³ KNO₃ c.2 grm.

NaNO₃ 0.7 "

KH₂PO₄ 0.1 "

MgSO₄ 0.1 "

CaSO₄ 0.1 "

NaCl 0.1 "

Fe₂Cl₆ 0.04 "

Distilled H₂O 1 litre.

⁸ Ibid., p. 193.

⁴ Physiol. Res., i, p. 394.

⁵ Ibid., p. 158.

⁶ (NH₄)₂SO₄ 0.94 grm.

K₂HPO₄ 0.2 "

MgSO₄ 0.45 "

CaCl₂ 0.1 "

FeSO₄ 0.0025 "

Distilled H₂O 1 litre.

⁹ Amer. Journ. Bot., x, p. 555.

alkalinity and the presence of nitrogen in the form of ammonium sulphate, instead of the more usual nitrate. The plants in this solution, both with and without boric acid, blackened and died before the conclusion of the experiment, in a manner quite distinct from the typical withering due to a deficiency of boron. One plant, however, which had not received boric acid, exhibited the typical symptoms of a lack of boron in spite of its early death, thus showing that a supply of boron was probably as necessary in this medium as in the Rothamsted solution, but that its utility might be masked by the presence of a toxic factor.

In all the other solutions the plants died characteristically unless boron was supplied. The time elapsing before the withering set in was much the same in every case, although the amount of growth made before death varied with the different nutrient media. Since the culture solutions tested were of such widely different composition, and since in all cases a supply of boron was found necessary for the broad bean, it seems probable that any variety of culture medium, not being definitely toxic, would yield similar results, and confirm the view that the effect of the presence or absence of boric acid is independent of the nature of the nutrient solution.

TABLE I.

Average Total Dry Weight of Single Broad Bean Plants grown in Different Nutrient Solutions with and without Boric Acid, June 2 to July 16, 1924.

Solution.	Dry Weights. gram.	
	+ B.	— B.
Crone	12.27	4.22
Rothamsted (pH 6.2)	11.80	5.39
Detmer	9.83	2.15
Allison and Shive	6.99	5.84
Tottingham $T_3R_1C_4$	6.07	4.67
Knop	6.64	3.86
Modification of Rothamsted (pH 3.8)	5.88	2.65
Shive R_5C_2	5.55	5.01
Tottingham $T_1R_7C_1$	5.52	3.74
Modification of Moore	2.11	1.83

2. INFLUENCE OF CONCENTRATION AND TOTAL SUPPLY OF BORIC ACID ON GROWTH.

With *Vicia Faba* all concentrations of boric acid down to 1 : 2½ millions, other than those which are definitely toxic and check growth, have repeatedly been found effective in promoting healthy growth of the meristematic apices, even though the strength may be sufficiently great to cause symptoms of poisoning in the lower leaves. The influence of lower concentrations was approached in correlation with the question as to whether the determining factor is the actual concentration of the boron compound present at any one time, or the total amount of boron supplied over any given period, irre-

spective of its concentration. In one of two parallel series of plants, with boric acid ranging from 1:80 millions to 1:2½ millions, with a no-boron control (five similar plants being the unit), the nutrient solutions were renewed as is usual in this experimental work, the intervals between changes being from sixteen days in the earliest stages to eight days as the plants developed ('normal change'). In the other series the change was made four times as often ('frequent change'), the plants in this way receiving four times as much boron as the others, any one concentration thus being parallel to one four times as heavy in the normal change as far as quantity of boron is concerned.

With the *normal change* signs of boron deficiency appeared as usual in about three weeks where boron was entirely withheld, and soon after manifested itself with 1:80 millions. With increase in boron the symptoms appeared later, 1:10 million being apparently on the border-line of efficiency (Pl. X, Fig. 3). In the latter case some plants remained quite healthy to the end, others showed characteristic deficiency symptoms, while one plant appeared to recover from incipient boron starvation when fresh solution was given, and to show signs of deficiency shortly before the next change was due. The height of the main stem increased rapidly with the boron, due to the extra growth that was made before the death of the apices. The formation of lateral shoots was much promoted by insufficient boron, being a natural response of the plant to the death of the apices, as occurs with mechanical injury. The relative number of dead or unhealthy tillers was also in inverse proportion to the supply of boron, the few tillers with 1:2½ millions boric acid being entirely healthy, in contrast to thirty-two unhealthy tillers out of thirty-six with 1:40 millions boric acid for the same number of plants (Table II).

TABLE II.

*Dry Weight of Vicia Faba. Average of Five Plants,
July 2 to Sept. 24, 1925.*

Concentration of Boric Acid.	Nutritive Solution changed	
	Normally. gram.	Frequently. gram.
None	6.78 ± 0.89	19.21 ± 0.73
1:80,000,000	9.19 ± 0.44	19.77 ± 1.89
1:40,000,000	12.20 ± 0.63	24.65 ± 3.14 *
1:20,000,000	13.26 ± 0.82	32.68 ± 0.61
1:10,000,000	16.13 ± 1.01	21.16 ± 1.40
1:2,500,000	17.32 ± 0.62	28.04 ± 1.47

* Average of four plants only.

The total dry weight rose to between two and three times its value from no boron to 1:2½ millions, but the most noteworthy point is the considerable increase due to 1:80 millions boric acid. This concentration represents the supply of 0.0675 mg. boric acid throughout the three months

of the plant's life, and it can only be regarded as amazing that such a small amount, insufficient to postpone the death of the growing-points for more than a few days, should yet suffice to enable the plant to lay down such a large increase in dry material. This fact, together with the alternating behaviour of one of the plants receiving 1 : 10 millions boric acid, suggests that the broad bean is able to utilize effectively even the smallest traces of boron, and that if it were possible to replace this small amount as fast as it was used up, no trace of boron deficiency would manifest itself, however low the concentration at any particular moment. In other words, the critical factor is the absolute amount of boron available in any given period, and the concentration is immaterial so long as the rate of replacement is rapid enough to supply the requisite total amount.

This idea received support from the *frequently changed* series. The more frequent renewals entailed the supply of much additional food as well as of boron, resulting in the production of much larger plants. For some reason, as yet unexplained, the signs of deficiency in the absence of boron were considerably delayed, an extra week elapsing before any suspicious symptoms appeared. Even then their development was slow and the plants continued to make much growth, but both main and side shoots ultimately died with the usual characteristic appearances. More laterals were produced in this case than in any set receiving boron, and this heavy shoot production probably accounts for the fact that the dry weight without boron is practically the same as with 1 : 80 millions boric acid, in which few laterals occurred (Table II). In this latter case, which represented a total of 0.27 mg. boric acid during the life of the plant, the deficiency signs were similarly delayed and they progressed still more slowly, some apices remaining healthy or merely suspicious to the end, others dying characteristically. Of the small number of side shoots formed, most flowered normally and few were unhealthy. With more boric acid, 1 : 40 millions, suspicious signs of deficiency were noticed at the end of the first month, but soon disappeared, the plants developing normally, flowering and producing as much dry material as with larger quantities of boron (Pl. X, Fig. 4). This set corresponds as to amount of boron with the 1 : 10 millions normally changed, which was on the borderline of efficiency, but the extra food supply has in some way enabled the plants to throw off the initial harmful effects of boron deficiency, and to hold their own with others receiving a larger supply.

The general result emerging from the above experiments is that the concentration of the boric acid present is of little moment provided that an adequate total supply is available over a given period, but that this total supply is somewhat lessened when the nutrient solution is frequently renewed. The delay in the appearance of symptoms of boron deficiency in frequently changed solutions needs further investigation by means of drip cultures in which the balance of the nutrient salts is maintained at a con-

stant level throughout, but initial experiments of this type carried out in 1925 were conducted too late in the year to give satisfactory results.

3. NON-INTERCHANGEABILITY OF BORON AND OTHER ELEMENTS.

Although boric acid had proved so consistently favourable to the growth of *Vicia Faba*, it had not been sufficiently demonstrated that the boron was of necessity the active principle of the compound. This problem was therefore approached from two sides, to ascertain:

- (a) whether boron is equally effective when applied in combinations other than boric acid;
- (b) whether any other element is able to supply the needs of the plant in the same way as boron.

(a) *Effect of various boron compounds.*

In all cases the compounds tested were utilized in such quantity as to supply the same amount of boron as that contained in a solution of one part per million of boric acid, the latter being used as a standard of comparison, in addition to controls without boron. The test substances were added to the ordinary nutrient solution (pH 6.2), which was changed once or twice during growth. The soluble borates of sodium (mono- and pyro-borate), lithium, and potassium presented no difficulty, but the insoluble or very slightly soluble borates of aluminium, calcium, cobalt, copper, lead, magnesium, manganese, nickel, strontium, and zinc needed special treatment. The requisite quantities of the latter were weighed out and ground up as finely as possible in a crucible before adding to the solutions.¹ It seemed feasible that even the so-called insoluble borates might dissolve in the small quantities necessary to give concentrations equivalent to 1:1,000,000 boric acid, and this assumption was justified by the results. In three weeks the effects were clearly marked. All plants receiving either soluble or insoluble borates were as good as those with boric acid, being healthy at the apex and having well-developed flower-buds, while the controls showed the characteristic signs of boron deficiency (Pl. X, Fig. 5). At harvesting all the plants supplied with boron were tall and in full flower, whereas those without any were dead at the shoot apex, and exhibited dead undeveloped flower-buds and stunted root growth. While the borate plants were obviously receiving a sufficiency of boron they showed certain differences among themselves in the total amount of growth made, a phenomenon that was probably due to a slightly toxic or stimulant action exercised by the various compounds. This was specially noticeable with calcium, cobalt, and sodium mono-borate, which tended to depress growth, and with manganese borate, which yielded the heaviest plants of all. The appended

¹ This experiment was set up and started by Dr. G. H. Duff, of Toronto University.

weights, associated with the character of the growth made, point clearly to boron itself as being the active agent which enabled normal and complete development to take place. The plants were harvested as soon as signs of boron deficiency were distinct. Consequently the differences in dry weight between those with and without boron are less striking than in other cases where growth extended over a longer period.

TABLE III.

*Dry Weights of Vicia Faba grown with various Boron Compounds.
Average of Five Plants, Aug. 2 to Sept. 15, 1924.*

		Shoot. gm.	Root. gm.	Total. gm.
Control. No boron	2.66	0.52	3.18
Boric acid	4.11	0.93	5.04
<i>Soluble borates.</i>				
Lithium borate	3.96	0.73	4.69
Potassium borate	4.29	0.76	5.05
Sodium mono-borate . .	.	3.57	0.62	4.19
„ pyro-borate	4.36	1.00	5.36
<i>Insoluble borates.</i>				
Aluminium borate	4.36	0.75	5.11
Calcium „	3.54	0.64	4.18
Cobalt „	3.26	0.74	4.00
Copper „	4.55	0.69	5.24
Lead „	4.14	0.61	4.75
Magnesium „	4.46	0.77	5.23
Manganese „	5.46	1.14	6.60
Nickel „	4.12	0.77	4.89
Strontium „	3.73	0.74	4.47
Zinc „	3.83	0.79	4.62

The above results received additional support from tests with a few organic compounds. With glycerine, magnesium citrate, and citric acid, growth in every respect resembled that with no boron, whereas with boro-glycerine and sodium boro-citrate the plants corresponded to those receiving boric acid (Pl. X, Fig. 6). It has already been shown (3, p. 647) that borax and boric acid are alike in their effect on the growth of barley, and it now seems probable that with all plants for which boron is essential the form of its presentation is immaterial, and that sufficient can be abstracted for normal growth even when it is present in a relatively insoluble form.

(b) Possibility of replacement of boron by other elements.

The failure of various compounds, added to the nutrient solutions at various times, to induce normal growth of *Vicia Faba*, unless they contain boron, led to a systematic investigation of many other elements to ascertain whether any of them could function in the same way as boron. Owing to limitations of space it was necessary to deal with the elements in several batches over a period of three years, and up to date fifty-two have been

tested. The remainder are chiefly the rarer elements which it is difficult or almost impossible to obtain on the score of expense or scarcity, or are gases with which it is not easy to deal, even when they are available in sufficient quantity.

PERIODIC TABLE.

Group O.	I.	II.	III.	IV.	V.	VI.	VII.	VIII.
	H							
<i>He</i>	Li	Gl	B	C	N	O	F	
<i>Ne</i>	Na	Mg	Al	Si	P	S	Cl	
<i>A</i>	K	Ca	<i>Sc</i>	Ti	V	Cr	Mn	Fe Co Ni
	Cu	Zn	<i>Ga</i>	<i>Ge</i>	As	Se	Br	
<i>Kr</i>	Rb	Sr	Yt	Zr	<i>Cb</i>	Mo		<i>Ru Rh Pd</i>
	Ag	Cd	<i>In</i>	Sn	Sb	Te	I	
<i>Xe</i>	Cs	Ba	La	Ce				
			<i>Other rare earthelements.</i>		<i>Ta</i>	W		<i>Os Ir Pt</i>
	Au	Hg	Tl	Pb	Bi			
<i>Nt</i>		<i>Ra</i>		Th		U		

Bold type in the above table indicates the elements which have been tested to date, and for the sake of clearness those not tried are inserted in italics.

The tests were made with any convenient compound, frequently chloride or nitrate, utilized in such quantity as to supply an amount of the element in question equivalent to that of the boron in boric acid of concentrations of 1 and 5 parts in $2\frac{1}{2}$ million parts of solution, equivalence being based on the relative atomic weights of boron and the element. The nutrient solutions were renewed at the ordinary intervals, and by using both strong and weak solutions of the compounds it was anticipated that any difference in the range of activity of the various elements would be covered.¹ In no single instance, however, was any element found to

¹ Some of the elements, **Al**, **Mn**, **Cs**, **Rb**, **Li**, **Th**, and **Zn**, were tested by Miss D. Marx, B.Sc.

replace boron (Pl. X, Fig. 7). As a general rule the plants receiving other elements began to show the characteristic signs of boron deficiency at the same time as those to which no addition had been made. This set in after three to five weeks from the start, but plants grown early in the year or in autumn developed satisfactorily without boron for rather longer.

A few elements proved distinctly toxic at the concentrations used, and in these cases the deficiency signs were occasionally delayed in appearance. Mercuric chloride apparently killed the plants from the outset in both concentrations, but after ten days those in the weak solutions began to recover, and still later some of those in the stronger solutions started into growth. Under these circumstances the earliest signs of boron deficiency did not appear for three weeks after they were well marked with other elements, probably owing to the supply of boron in the seed not having been used up during the preliminary period of paralysis. A rather similar effect, though less marked, was observed with gold chloride, especially in the strong solution; in the weak solution the ultimate recovery brought the plants up to the level of the controls without boron. Cobalt and cadmium sulphate behaved like gold in both concentrations, retarding the appearance of the signs of boron deficiency, and they also reduced the dry weights of the plants in strong solutions. The effect of tellurium nitrate was unusual, in that the strong concentrations were so toxic as to kill the plants at the outset, whereas the weak strength produced no symptoms of poisoning throughout, the plants growing normally and exhibiting signs of boron deficiency at the usual time. The strong concentration of vanadium chloride was also definitely toxic, inducing a very characteristic type of short root, with stiff and stubby laterals. The shoots were rather long and spindly, and began to flower before dying of boron starvation, which did not appear for about seven weeks. The weak strength retarded growth for a while, but ultimately the plants caught up the controls and showed the effects of lack of boron at the same time. In view of the possibility that the valency of the element might influence matters, copper was used both as cuprous chloride and copper sulphate, with identical results in each case. The weight of evidence, therefore, indicates that boron occupies a unique position in the nutrition of certain plants, in that in some way or other it affects the development and functioning of the meristematic tissues (3, p. 668) and is irreplaceable by any other element. This action may be direct or indirect, i. e. boron may either act directly as a nutritive element essential in itself, or indirectly by influencing the intake or utilization of other nutritive elements that are needed in larger proportions. To gain some light on this question various nutritional experiments were undertaken, as hereafter described (p. 181), but much remains to be done before the true mode of action of the boron can be elucidated.

4. RELATION OF BORON TO THE GROWTH OF VARIOUS SPECIES.

(a) *Species tested.*

The first striking results with boron were obtained with the broad bean, only a few small scale tests being made with other species. More recently the effect of boric acid has been tried on a large variety of plants to determine which species, if any, also require boron to complete their development. The results have confirmed and extended those previously obtained, the plants tested being divisible into three main groups:

- A. Those for which boron is undoubtedly essential for normal growth.
- B. Those on which boron may have a beneficial effect, but for which it is not essential.
- C. Those for which the results are inconclusive, including those cases where the plants failed from unknown causes in the early stages.

A. The necessity for the addition of a small quantity of boric acid to the nutrient solution has been clearly shown and frequently confirmed in the case, of soy bean (*Glycine hispida*) (Pl. XI, Fig. 8), scarlet runner bean (*Phaseolus multiflorus*), and crimson and red clover (*Trifolium incarnatum* (Fig. 9), and *T. pratense*). All these species died at the apex of the shoot, failed to flower, and developed stunted roots unless boron was supplied. Equally striking evidence was obtained of the necessity of boron for yellow and wild white clover (*Trifolium minus* and *T. repens*), melon (*Cucumis Melo*) (Pl. XI, Fig. 10), and a variety of broad bean other than Sutton's Prolific Longpod, viz. Green Windsor. No confirmatory tests were made with these latter species, as the characteristic withering of the growing-point and failure of the flowers to develop seemed sufficient proof. At least five plants were grown both with and without boron in each case, and all those similarly treated yielded entirely consistent results. The importance of carrying on the plants until the flowering stage is reached was emphasized in the case of wild white clover, which had previously been classed as a plant benefiting from boron, but not dependent on it (3, p. 666). Repetition and extension of the duration of the experiment, however, showed that the earlier result was incomplete, as stunting of the roots, death of the shoot apices, and failure of the flowers occurred eventually where boron was withheld, whereas if supplied with boron the plants flowered freely and continued growing for many months. In all probability lucerne (*Medicago sativa*) belongs to this group, as the plants without boron were poor and showed a distinct tendency to develop the characteristically stunted root system, but conclusive evidence at the flowering stage was lacking, as all plants were severely injured with aphid infestation.

B. Peas, of which three varieties have been grown, barley, and candy-tuft (*Iberis umbellata*, var.), are all able to complete their normal develop-

ment, including flower and fruit formation, in the entire absence of boron, although in some cases they show a distinct benefit from the addition of small quantities of this element (Pl. XI, Fig. 11).

C. Maize, ten-week stock (*Mathiola* sp.), and spinach all showed a definite improvement in vegetative growth if supplied with boron, but owing to the intervention of unsuitable seasonal conditions it was not possible to carry on the experiment sufficiently long to determine whether or not boron is essential. The results with buckwheat, potato, and *Convolvulus minor* were variable and therefore inconclusive. Other species tested, viz. sweet pea, yellow and white lupin, sunflower, aster, and chrysanthemum, failed to grow satisfactorily in the culture solution used, and so yielded no results.

In all cases, whether species requiring boron are long- or short-lived, the necessity becomes more manifest as the flowering period approaches. This may account for the apparent anomaly of the boron present in small seeds, such as clover, being sufficient to carry on the plant for several months, whereas that in much larger seeds also well supplied with boron, as broad bean, is insufficient for as many weeks. It would be as well to emphasize here that, in the case of all plants for which boron has been claimed as essential, the meristematic apices of the shoot actually die, usually as the flowering period approaches, unless this element is supplied. The effect of boron in preventing this premature death as in A above must be sharply distinguished from mere vegetative improvement or 'stimulation' brought about by its addition as in B, where the control without boron remains quite healthy although less strongly developed in comparison. These two phenomena have been frequently confused and claims made for the necessity of an element without evidence of actual death in its absence having been obtained. McHargue (2), however, in his work on manganese, has described the dying back of young branches of soy beans in sand cultures from which this element was carefully excluded. This result is of special interest in view of the present investigation, the results of which show that boron is essential for the complete development of the same plant. The possibility of these two elements being mutually replaceable was therefore tested, although in the case of the broad bean this view has been shown to be untenable (p. 175). Small amounts of manganese sulphate were added to the nutrient solutions of soy bean plants, which for several weeks previously had been grown in water culture, both with and without boric acid. The addition of manganese was entirely without effect on the plants deprived of boron (Fig. 12, right), which by this time were beginning to die characteristically at their shoot apices, whereas the supply of boric acid to similar plants at the rate of one part in two and a half millions of solution (containing boron equivalent to the manganese used above) caused a renewal of healthy growth, first apparent in the root system and later also in the

development of lateral buds in the shoot. The two elements are therefore distinct in function, and, as in the nutrition of the broad bean, they are evidently not interchangeable. Soy beans, however, supplied with boron throughout their life, and with manganese also in the later stages, were decidedly superior, especially as regards colour, to those receiving boron only (Pl. XI, Fig. 12, left). Manganese therefore exerted a stimulating influence, but did not appear to be essential for the nutrition of the soy bean in the same sense as boron. It is possible that a mere trace of manganese may have been introduced into the nutrient solution in the first few weeks of the experiment by the use of ferric chloride which had not been spectroscopically examined, as were all the other nutritive salts, although in the later stages of growth a specially prepared Mn-free salt was substituted. If manganese is essential, the trace thus supplied at first must have been adequate for the needs of the plant. This, however, does not alter the fact that healthy growth could not be obtained in the complete absence of boron. The normal and well-developed plants obtained by McHargue after supplying manganese, without the conscious addition of boron, would therefore suggest the presence of a trace of this latter element, sufficient for the needs of the soy bean, as an impurity in the sand or salts he employed. There is, of course, nothing to preclude the possibility that both manganese and boron are necessary for complete and normal development of this and other plants, but that the presence of a trace of the one element is needed before proof of the dependence of the plant on the other can be obtained.

(b) *Consideration of possibility of general need of boron for nutrition.*

It has been shown in the present investigation, employing wide limits of concentration of boric acid, that a sharp distinction can be drawn between plants for which boron is essential and those for which it is beneficial only. The possibility, however, is not precluded that, if still lower concentrations could be achieved, the latter class might merge into the former, the differentiation between the two classes proving to be simply a matter of degree in their boron requirements.

Under any given set of conditions, since death of the meristematic apices is the criterion upon which the results are based, the line of demarcation between species requiring or not requiring boron is necessarily sharp, but it is possible that under other conditions, e.g. with methods of greater or less refinement, the classification of the species might need modification. The broad bean is an instance in which the necessity of a supply of boron can readily be demonstrated, yet 0.05 mg. boric acid per week for a single individual is sufficient to obtain this effect in a well-marked degree. It is therefore conceivable that other species, e.g. pea, hitherto classified as not dependent on a supply of boron, may in reality need it, though in much less quantity than such plants as the broad bean. The amount necessary might

be so small that the boron normally present in the seed, or supplied from such outside sources as by solution from the glass culture bottles, would be sufficient to prevent the appearance of deficiency effects. Spectroscopic examination of solutions left standing for some weeks in the bottles, however, revealed no trace of boron, the methods adopted by Dr. Judd Lewis being sufficiently refined to indicate the presence of 1/1000 mg. boron in 100 mg. of solid matter, which is equivalent to detecting less than 1 part of boric acid in 180 million parts of the culture solution employed in these experiments. As it has thus been proved that no boron is received from this source, that supplied by the seed appears to be all that is available. The fact that viable seed is produced from peas grown in water culture enables this point to be investigated. With no external source of supply, the quantity of boron in any one seed would be progressively reduced by distribution amongst the seeds of successive generations. If boron is indeed necessary for the pea, a point would inevitably be reached when the share received by any one seed would be insufficient and signs of boron starvation should ensue. If, however, boron is not necessary, there seems no reason why healthy growth and seed production should not proceed for numerous generations. In this connexion it is interesting to recall that Dr. Lilian J. Clarke, at Dulwich, grew sweet peas in water culture for fourteen successive generations without the addition of boron. This, however, cannot be regarded as a conclusive proof that the sweet pea is independent of boron, since the nutritive salts used were not specifically tested for the absence of this element.

5. PHYSIOLOGICAL FUNCTION OF BORON IN THE NUTRITION OF *VICIA FABA.*

(a) *Non-replacement of nutritive elements by boron.*

As indicated above, boron may affect nutrition in either a direct or indirect manner, but it seemed necessary to approach the former standpoint circuitously. It is obvious that boron is essential to growth, but it is not so obvious whether the phenomena exhibited in its absence are due in reality to boron starvation or to deficiency of some nutritive element whose supply or utilization is contingent upon the presence of a sufficient supply of boron. It was conceived that, if the latter were the case, similar signs of deficiency, e. g. death of stem apices and of flower-buds, would occur if the element in question were absent from the nutrient solution even in the presence of an adequate supply of boron. Furthermore, if this did not occur, and if the characteristic phenomena exhibited in the absence of any element appeared both in the presence and absence of boron, clear evidence would be obtained that boron is unable to replace any of the other nutritive elements, but has a function peculiarly its own. To this end, therefore, a series of nutrient solutions was arranged of which the general composition, based on the

ordinary solution used, was kept as similar as possible contingent only upon one element being omitted from each. The pH values ranged from 6.1 to 6.6, except in the absence of chlorine, pH 5.5, and of phosphorus, pH 3.5. Broad beans were grown in each solution without boron and with 1 : 2,500,000 boric acid.

The absence of *sodium* or *chlorine* had apparently little effect on growth, as the plants closely resembled those grown in complete nutrient solutions, with and without boron, though without sodium and with boron the dry weights were slightly reduced. The omission of *sulphur* reduced the dry weight in both cases, and with boron the plants were yellowish, growth being otherwise similar to that with full nutrients. Without *nitrogen* a greater differentiation was noticeable, for, whereas with boron the plants soon became weedy and yellowish as the nitrogen in the seed was rapidly exhausted, without boron they remained green almost to the end, owing to the slower utilization of the reserve nitrogen. In each case, again, the appropriate signs of the presence or absence of boron were evident at the usual periods. *Iron* deprivation was shown by chlorosis of the upper leaves and top of the stem, associated with a reddish-brown spotting of surface and edges of leaves, which afterwards turned black and in some cases withered. But, although this injury essentially involved the upper part of the plant, it could not possibly be confused with that due to boron deficiency, as in the presence of boron the apex remained functional, however chlorotic it became, and the plants flowered well, whereas without boron the apex withered in the ordinary way and the flower-buds failed to develop. The chlorosis did not proceed so far without boron, on account of the slackening in speed of growth which prevented the shortage of iron, over and above that supplied by the seed, from becoming so acute. In the absence of *magnesium* the plants were weak and yellow, showing reddish-brown blotches on many leaves, though not at the extreme apex. With boron the apices remained healthy and flowering occurred, but without boron signs of deficiency began to show rather later than usual and did not progress so rapidly, apparently owing to the very poor growth, suggesting that the magnesium deficiency was predominant over the boron deficiency. With regard to *potassium* the opposite nature of the two deficiency factors was strikingly marked. The absence of potassium induced injury of the lower leaves, which appeared as if rubbed or pinched, became blackened, and eventually withered and fell off. With boron absent as well, the usual deficiency phenomena appeared at the apex, but in its presence the apex remained alive, although in most cases only a few leaves remained thereon at the end, owing to the extreme potassium starvation. The difference in the types of deficiency was further accentuated in several plants receiving boron in which the main shoots had died *from the bottom upwards*, each having, however, a lateral branch still partially healthy and in flower.

Without *phosphorus* growth was most severely checked, the plants being small, very dark green in colour, with very small leaves and poor roots. So slow and weak was growth that even without boron some plants came into flower, presumably by virtue of the non-exhaustion of the boron in the seed, but eventually even these showed the characteristic deficiency signs at the apex. The roots with boron were somewhat better than those without, and the shoot apices remained healthy to the end. The omission of *calcium* had such a drastic effect that very little growth was made before the plants turned black and died, long before the symptoms of boron deficiency could manifest themselves. The relative effect on growth of the omission of individual elements is shown by a comparison of the dry weights (Table IV), together with the superimposed effect of the omission of boron from the solution. In every case except magnesium, the characteristic signs of boron deficiency were correlated with a further decrease in weight over and above that caused by the omission of the element concerned. With magnesium omitted, the dry weights were the same under both conditions, suggesting a correlation with the late appearance of boron deficiency owing to the predominant action of magnesium deficiency.

TABLE IV.

Dry Weights of Vicia Faba grown with Omission of Various Nutritive Elements.

	+ B. grm.	— B. grm.
Complete .	12.82	5.15
No chlorine .	12.77	5.04
No sodium .	11.63	5.14
No sulphur .	9.79	3.74
No iron .	7.34	5.15
No nitrogen .	5.66	3.56
No magnesium	4.62	4.60
No potassium	4.47	3.49
No phosphorus	1.91	1.36
No calcium .		

It is therefore evident that, except for the unproven case of calcium, the particular phenomena induced by the omission of each element appear independently of the presence or absence of boron, showing that the latter is not able to replace any one of the ordinary nutritive elements. Furthermore, none of the signs hitherto associated with boron deficiency ever appeared with the omission of any element when boron was present, indicating that they are definitely associated with the presence of boron and do not indicate deficiency of any other element. The undecided case of calcium, however, was of the greatest importance in view of the fact that various slight indications had occurred that a possible connexion between calcium and boron might exist, and this matter was therefore approached from other standpoints.

(b) Effect of boron on growth in individual food salts.

In view of the blackening and death of the plants grown without calcium, implying toxicity of the food solution which is neutralized by the presence of calcium, the individual food salts were tested in solution at the same rate at which they are used in the compound solution, to determine which one, or more, brought about this characteristic type of dying, and whether it could be modified or prevented by the addition of boron. Ferric chloride killed the plants forthwith. Magnesium sulphate and sodium chloride caused injury at a very early date, hardly any shoot developed, and blackening of the whole plant rapidly set in. Potassium nitrate, potassium di-hydrogen and mono-hydrogen phosphate were progressively a little later in causing injury, and in all cases the shoots, where developed, eventually blackened. With each of these food salts death occurred without any difference being manifested by the presence or absence of boron. With calcium sulphate, on the other hand, growth was better and more rapid at first than in the complete nutrient solution, and signs of boron deficiency set in several days earlier than in the latter case. It was only later on, when the absence of other food salts made itself felt, that the calcium plants fell behind the complete nutrient set in development, but to the end those with boron were strikingly good, and those without showed all the signs of boron deficiency most markedly. In distilled water alone, however, all the plants began to die three weeks earlier than the calcium and complete nutrient sets. The manner of dying, even where boron was present, at first suggested boron deficiency, but later the symptoms developed differently and the stem became blackened and limp some distance behind the tip (Pl. XI, Fig. 13), death apparently being due to starvation. It has already been shown (p. 181) that starvation does not affect the upper part of the plant first except with iron and possibly in the unproven case of calcium. As chlorosis did not appear here, showing that deficiency of iron was not the cause, further investigations were made with regard to calcium.

(c) Possible association of boron with calcium in plant nutrition.

Broad beans grown with the usual quantities of all food salts save calcium sulphate, which was only supplied at the rate of 0.025 gramm. per litre, made very poor growth with very small blackened leaves in the absence of boron, and within twenty-four days had turned black at the tips and withered back along the stem. The blackening was due to the toxic effect of the other food salts present in considerable excess over the calcium sulphate and the withering was the same as that observed in distilled water. These plants, therefore, were suffering so badly from calcium starvation that they died, as they were not obtaining sufficient calcium from the nutrient solution to counteract the harmful action of the other food salts.

Even the calcium in the seed was probably not used up, as death ensued before the food reserves were exhausted. Parallel plants receiving boric acid behaved quite differently, growing fairly tall and developing much better leaves, though these were somewhat blackened at the edges (Pl. XI, Fig. 14). Healthy flower-buds were developed, the stems did not blacken, and even after seven weeks' growth were still erect and unwithered, in striking contrast to the plants without boron. In both cases the same amount of calcium was supplied, but whereas without boron this appeared to be insufficient to prevent death from poisoning by other food salts in excess, with boron it was sufficient not only to do this but to enable the plant to proceed with development of healthy flower-buds. This suggests that without boron the calcium is not fully utilized, and that in some way boron either enables the plant actually to absorb more calcium in a given time, or to utilize it more efficiently in metabolism when once it is within the plant. The solutions were occasionally renewed during growth, and immediately after a change the newly formed leaves showed scarcely any blackening, owing to the fresh supply of calcium, but as time went on and the calcium was used up the discoloration gradually appeared and became worse in the young leaves until another renewal was made, when the same phenomenon again occurred. When the plants grew larger, however, the amount of calcium sulphate supplied at the normal rate of renewal of solution was insufficient for the needs of the plant, and the withering of the stem due to calcium starvation made its appearance (Pl. XI, Fig. 15). With increasing amounts of calcium sulphate in the solution the signs of calcium starvation without boron became less marked until with 0.1 grm. or more CaSO_4 per litre they practically disappeared and their place was taken by the typical symptoms of boron deficiency (Fig. 14). This suggests that even in the presence of an abundant supply of calcium at the roots it cannot be efficiently utilized in the absence of boron, the injury in this case being localized in the meristematic apices. With a shortage of calcium the characteristic signs of boron deficiency in the shoot are marked by the more dominant symptoms caused by the toxic action of the other food salts. This masking does not occur in the root, but even with as little as 0.025 grm. of calcium sulphate per litre the roots are of the typical 'no-boron' type, being short and thickened, whereas with boron present they are much longer and thinner, as is usual. These results were corroborated by frequent repetition of experiments, variations being made to clear up debatable points as they arose. Although the association between boron and calcium appears to be close, the two elements are not mutually replaceable, as was further proved by growing plants without boron and with very little calcium for some time and then supplying one or both elements. Boron was of little use if the calcium deficiency was not made good, and the plants continued to blacken and die, whereas extra calcium encouraged a certain

amount of leafy growth, after which death occurred with the typical signs of boron deficiency. In both these cases the ultimate dry weight of the plants was very similar. With boron and extra calcium recovery set in and healthy growth resulted, giving, by the time the experiment was closed, more than double the dry weight obtained in the absence of either calcium or boron (Table V).

TABLE V.

Mean Dry Weights of Vicia Faba. Grown for Fifty-three Days.

All initially treated uniformly for seventeen days with no boron and 0.025 gm. CaSO_4 per litre nutrient solution.

<i>Treatment after Seventeen Days.</i>	<i>Shoot.</i> gm.	<i>Root.</i> gm.	<i>Total.</i> gm.
No addition	1.39	0.19	1.58
Boric acid added (1 : 1,000,000)	2.46	0.46	2.92
Extra CaSO_4 added (0.1 gm. per litre)	2.81	0.48	3.29
Extra CaSO_4 and boric acid added	6.30	1.30	7.60

In this connexion it may be well to recall the fact that, although so little boron is required for the well-being of the plant, a constant supply is necessary. Earlier experiments (3, pp. 642-66), with the addition and removal of boron at various stages of growth, showed that it was needed throughout for perfect growth to be made, indicating that the absorbed boron is definitely used in metabolism and in some way fixed by the plant. It is not therefore acting as an ordinary catalyst, an initial supply continuing in action over a long period, but is itself removed from the sphere of action in the course of its association with the functioning of calcium, other nutritive elements having apparently been ruled out. *How* the boron influences the utilization of this element remains unexplained, and further work will be directed towards the elucidation of this problem.

SUMMARY.

1. The need of certain plants for boron seems to be unaffected by the nature of the substratum on which they grow, the conditions of aeration at the roots, or, in the case of leguminous plants, the presence or absence of nodules thereon. In water cultures the necessity of the element is shown to be independent of the composition or pH value of the nutritive solution.

2. The concentration of boric acid appears to be of little moment provided that an adequate, though not excessive, total quantity is supplied over a given period, but this total supply is somewhat lessened when the nutritive solution is frequently renewed.

3. The chemical combination in which boron is presented to the plant is immaterial, even the so-called 'insoluble' borates being effective, but no other element, out of fifty-two tested, has proved capable of replacing boron. Special attention has been given to manganese in this connexion.

4. Boron has proved to be essential for several leguminous plants and for melon, whereas various cereals and candytuft complete their development in its absence. It remains to be proved whether the distinction between these two classes is real or merely a matter of degree, i. e. whether the second class require so little boron that a sufficient supply is stored up in their seeds.

5. The physiological function of boron in the nutrition of broad bean is under investigation. Boron is not able to replace any one of the essential nutritive elements, but a definite association with the absorption or utilization of calcium is very strongly suggested. The boron does not act as an ordinary catalyst, but is itself absorbed and in some way removed from action, a constant supply thus being necessary.

In conclusion, we wish to acknowledge our indebtedness to Dr. G. H. Duff, of Toronto University, and Miss D. Marx, B.Sc., for assistance freely given in connexion with certain of the experiments. Our thanks are also due to Mr. Martin Sutton for his liberality in supplying seeds year by year, and also to Professor Stapledon, Dr. Piper, Mr. J. W. Read, and the Seed Testing Station, Cambridge, for the gift of pure line seed of various species.

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ADDENDUM.

Since this paper went to press the question as to the possibility of preventing the characteristic death of plants grown without boron by frequent enough renewal of the nutrient solution (p. 172) has been tested by means of 'drip' cultures. Broad beans were grown singly in 600 cc. culture bottles and the apparatus adjusted so that the solution for each plant was entirely renewed every twenty-four hours. Similar plants received an addition of one part boric acid per million of solution. Growth in every case was exceptionally good, and all plants, whether receiving boron or not, flowered freely, although only those supplied with boron formed pods. Considerable delay occurred in the appearance of signs of boron deficiency. This delay varied from three to six weeks beyond the date at which plants grown simultaneously, but with solutions renewed at the usual intervals, first showed the symptoms. In spite of this, all the plants in drip cultures deprived of boron eventually died in the characteristic manner, whereas those supplied with boron remained quite healthy to the end of the experiment (eighty-five days in all).

It is evident, therefore, that a plentiful supply of nutrients and the maintenance of a constant balance in the food solutions encourages growth for a longer period before signs of boron starvation become manifest, but are not able to prevent the ultimate death of the plant from this cause.

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EXPLANATION OF PLATES X AND XI.

(Illustrating Dr. Brenchley's and Miss Warington's paper on the Role of Boron in the Growth of Plants).

(All plants grown in full nutritive solutions in water cultures except where otherwise stated.)

PLATE X.

Fig. 1. Broad beans grown in sand cultures. Left, with boric acid; right, without boric acid.

Fig. 2. Roots of broad beans grown in sand cultures. Right, with boric acid; left, without boric acid.

Fig. 3. Broad beans grown with increasing amounts of boric acid; solutions renewed at usual intervals. 1. No boric acid; 2. 1:80 millions boric acid; 3. 1:40 millions; 4. 1:20 millions; 5. 1:10 millions; 6. 1:2½ millions.

Fig. 4. Broad beans grown at same time and with same concentration of boric acid as in Fig. 3, but with solutions renewed four times as often.

Fig. 5. Broad beans grown with soluble potassium and lithium borates, and insoluble magnesium borate, compared with control plant without boron.

Fig. 6. Broad beans grown with, left to right, citric acid; magnesium citrate; sodium borocitrate; no boron (control).

Fig. 7. Broad beans grown with boron and various other elements; amounts used equivalent to boron in 1:2½ millions boric acid, compared with control without boron. (One typical photograph illustrating similar results with fifty-two elements tested.)

PLATE XI.

Fig. 8. Soy bean. Left, without boron; right, with 1:2½ millions boric acid.

Fig. 9. Crimson clover. Left, without boron; right, with 1:2½ millions boric acid.

Fig. 10. Melon. Left (two plants), without boron; right, with 1:2½ millions boric acid.

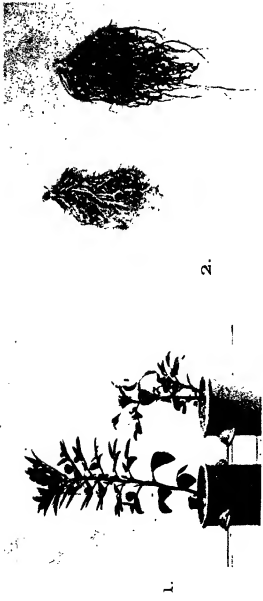
Fig. 11. Pea, var. Pioneer. Left, without boron; right, with 1:2½ millions boric acid, showing stimulation.

Fig. 12. Soy bean. Left to right, with boric acid throughout, manganese sulphate for last eight weeks; with boric acid, but no manganese; no boron throughout, manganese sulphate for last eight weeks; no boron and no manganese.

Fig. 13. Broad beans. Left, grown in distilled water only, without and with boric acid; right, grown in calcium sulphate solution only, without and with boric acid.

Fig. 14. Broad beans with varying quantities of calcium sulphate, but other food salts as usual. Left, without boric acid; right, with boric acid.

Fig. 15. Broad beans grown as in Fig. 14, but carried on till plant 2 showed calcium starvation.



1.

2.

NORMAL CHANGE



3.

FREQUENT CHANGE

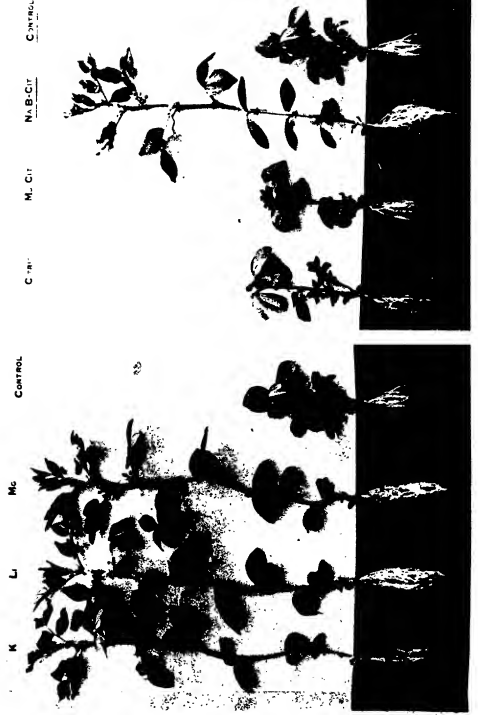


4.

INCREASING S



7.



5.



NOTES.

A BI-SPORANGIATE STROBILUS IN PSEUDOTSUGA.—In the course of a recent examination of ovules of *Larix* and *Pseudotsuga*, a bi-sporangiate strobilus was found among the cones collected. There is nothing now unique in the mere discovery of such a cone in the Pinaceae, although they are not very common; but, apart from the interest of the cone itself, it presented certain points which seem worthy of record. The stamens occupied the basal part, the ovuliferous area being confined to about the upper quarter. Now the normal staminate cone usually bursts from its winter scales clean, with no small leaves around its base as in the female cone; but here, although all the lower part was staminate, a few leaves, though smaller than in the normal female, were scattered up the pedicel shank below the lowest stamen. No grading existed between these leaves and the stamens, nor between the stamens of the lower part and the bracts and scales of the upper part. At the transition zone, however, a number of stamens bore one pollen-sac only, while some of the bracts had only one wing-like expansion from the characteristic central zone. In the axil, or apparently in the axil, of a few of the last stamens very small scales were carried, which, so far as could be seen, never carried more than one ovule. These ovules were rudimentary to the extent that, though the nucellus was well swollen, the integument was barely formed at all.

But the real interest of this cone attaches to the nature of the ovules carried in the upper, more typical, bracteate portion. To appreciate this, the peculiarities of the normal ovules of *Larix* and *Pseudotsuga* must be recalled. In a recent communication ('Proc. Roy. Ir. Acad.', vol. xxxvii, B, No. 19, 1926, p. 169) on the ovules of these two genera the writer has shown that, in the case of the ovule of *Larix*, one side of the integument, the side towards the cone axis, is extended out as a large flap, which curls over the micropylar mouth like a hood and has a very definite stigmatic function. It can be referred to as the stigmatic flap. In the case of *Pseudotsuga* the micropylar mouth, which is slit-like, is two-lipped, one lip, placed in the same way as in *Larix*, being very much larger than the other lip, which is an outgrowth of that side of the micropylar rim which is normally unchanged in *Larix*. The two are swollen and turgid—as is the single flap in *Larix*—and being pressed together are suggestive of the lips of an *Antirrhinum* corolla or rather of some minute Calceolarian form. In *Larix* the single large flap, subsequent to pollination, changes a little and eventually, as described in the communication, curls in on itself and thus introduces the pollen into the micropylar canal. In *Pseudotsuga*, although the two lips are present, only the large outer lip functions in the introduction of the pollen into the canal, the smaller lip being merely accessory. The large lip in *Pseudotsuga* is thus strictly comparable to the stigmatic flap of *Larix*.

If this be borne in mind the main interest attaching to the cone becomes obvious at once. In the axils of the first bracts of the typical ovuliferous portion of the cone scales were carried, much narrower than the normal, but bearing two ovules. In these, though a little better than in the rudimentary ones carried in the axil of a few of the stamens, the integument was poorly developed, consisting of a more or less irregular ring of tissue round the nucellus. But in the axils of the next succeeding bracts were carried scales, in size and shape apparently normal, and bearing two ovules. These ovules, however, never developed the two-lipped condition of the micropyle which is the character of *Pseudotsuga*. No matter how well grown, only a single large stigmatic flap was to be detected, situated, as in *Larix*, on the cone axis side, the rest of the micropylar rim being unchanged. None of the ovules in the ovuliferous part of cone showed the two-lipped condition. It is tempting to suggest that we have here, perhaps, an indication that *Larix* is, phylogenetically, the older form. There is evidence that *Larix* is an old genus; it may possibly have been developed by Wealden times; but speculation without data is futile. We have, however, further evidence in favour of the remarkably close relationship between *Larix* and *Pseudotsuga* upon which the writer has constantly insisted but which is not yet recognized by systematists.

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FERTILIZATION OF BRYOPHYTA. *Polytrichum commune*. (PRELIMINARY NOTE.)—From the researches of Pfeffer, Buller, Lidfors, and others it has long been known that sperms are attracted to the archegonia of Pteridophyta by malic acid or other chemotactic substance, that sugar is the attractive body in Musci and a protein in Hepaticae, provided that the living sperms are brought into the vicinity of the open necks of the archegonia; but, so far as we are aware, there is no record of how the sperms, in the first instance, reach the archegonia. As the result of prolonged observations on *Polytrichum commune* during spring and summer of the present year we have established the following facts:

1. Both the male and the female 'heads' are constantly visited by mites belonging to the group Oribatidae, two species of 'spring-tails' (Collembola), a small midge (Diptera), a larval form of one of the 'leaf hoppers' (Cicadidae), an Aphis, both winged and apterous forms, and a spider. (For the identification of these insects, &c., we are indebted to Professor R. Newstead, F.R.S., who has examined them *in situ*.)

2. Contrary to our original expectation we found that the paraphyses contained no sugar, but mucilage only. The mucilage was abundantly exuded, especially from the spatulate paraphyses on the male 'head', but also as well from the filiform paraphyses round the archegonia.

3. The visiting insects greedily lap this mucilage, while at the same time their bodies, legs, mouth parts, and antennae become smeared with the excretion. They

also lick at the saline crystals formed on the perichaetial leaf margins. They even pierce the antheridia themselves, for in some instances their intestines were found to be full of chlorophyll grains. The mucilage on their bodies contain large numbers of sperms, actively motile. In cases where the antheridial walls have been pierced, manifestly the apical dehiscing apparatus of the antheridium is unnecessary.

4. Archegoniophores yielded the same insects, whose bodies and limbs were also smeared with mucilage in which sperms were abundant.

5. The paraphyses surrounding the archegonia are well provided with mucilage in which sperms were frequently seen actively motile. The mucilaginous contents of the paraphyses have obviously the primary function of keeping the antheridia and archegonia moist, and secondarily, through the mediation of insect visitors, of facilitating the transference of sperms from the male to the female 'heads', often at a considerable distance from the male 'heads'. Further investigations are being made not only on other Musci but also on Hepaticae.

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D. MILLER-BROWN.

An Investigation of the Behaviour of Pectic Materials in Apples and other Plant Tissues.¹

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INTRODUCTION.

DURING the last few years a purely chemical investigation of the pectic constituents of apples has been carried out in these laboratories (9, 10, 11, 12). The evidence from this chemical study indicates that the pectic constituents occur in the apple in at least three forms: (1) *pectin*, a water-soluble substance which develops in the tissues during ripening; (2) *pectose*, an insoluble compound located in the cell-wall which, as ripening proceeds, appears to give rise to soluble pectin; (3) a complex containing pectic acid or a salt of pectic acid, of which complex the middle lamella is either partially or entirely composed. These three constituents have been extracted fractionally and estimated individually, and their relative proportions in the tissues determined.

During the progress of the chemical work, the value of a parallel microscopical study of the pectic changes in the cell-wall of the apple became evident. The results of such a microscopical¹ and micro-chemical investigation are here put forward, and an attempt has also been made to correlate the results obtained with those accruing from the purely chemical work.

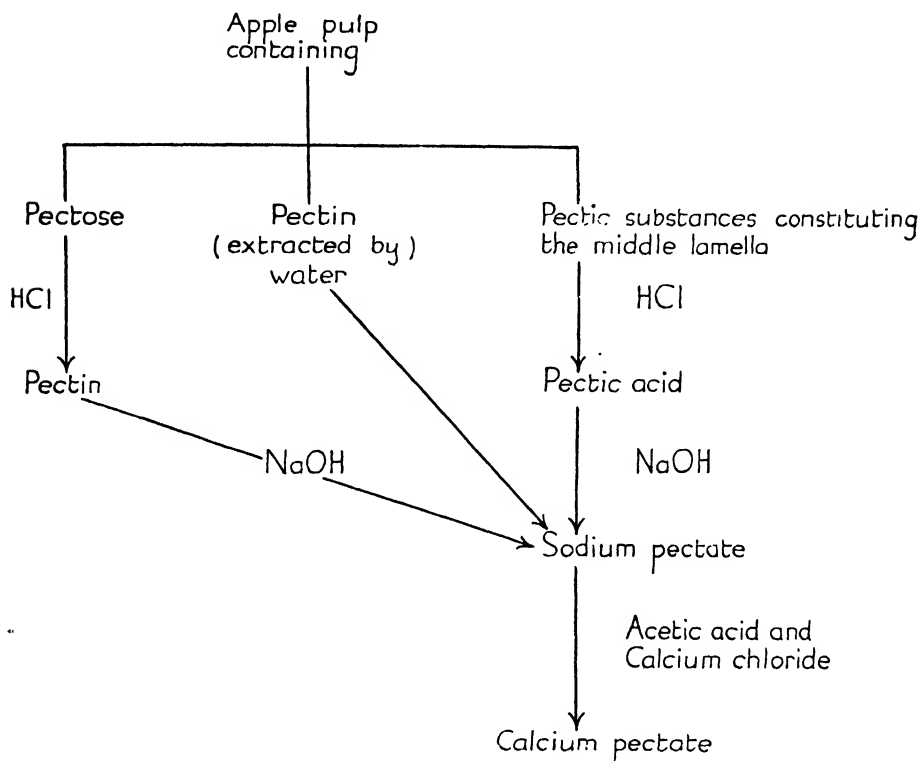
A brief statement will first be given of the results of the purely chemical study of the pectic changes, together with a brief description of the methods employed.

II. PECTIC CHANGES AS STUDIED BY CHEMICAL METHODS.

The chemical methods¹ depend upon the fact that the pectic compounds can be converted into an insoluble calcium salt of pectic acid of constant composition. A known weight of apple pulp is taken, and the soluble pectin is washed out with cold water and estimated as calcium pectate. The pulp is then treated with hydrochloric acid, and the pectose converted, presumably by a process of hydrolysis, into soluble pectin which is washed out as before and estimated. The residue is then treated to remove the remaining pectic substances constituting the middle lamella. The acid

¹ For fuller details see Carré (10, 11, 12).

treatment for removing the pectose simultaneously decomposes the middle lamella into free pectic acid. Pectic acid, however, is only partially soluble in dilute acid and in water, and cannot be extracted entirely as such from the tissues. By warming with dilute caustic soda, the pectic acid is con-



TEXT-FIG. 1. Diagram illustrating the extraction and estimation of pectic compounds.

verted into the water-soluble sodium salt, which may be removed from the pulp by washing and estimated by converting into the calcium salt. The accompanying diagram (Text-fig. 1) illustrates the processes of extraction¹ and estimation of the various pectic compounds in apple tissue.

These chemical methods were employed in tracing the changes in the pectic constituents of the apple from the stage of early ripening to the last stages of senescence and death, a period extending from September to the end of June in the following year, the fruit being held in cold storage at 1°C.

It was observed that, as ripening proceeds, there is a tendency towards

¹ Ammonium oxalate has been used by many authors, Mangin (38-44), Schryver and Haynes (52), &c., as a solvent for pectic substances. This reagent, however, removes all the various pectic substances. For this reason the use of this reagent was abandoned, since at the time it was desired to trace the relationship between the different pectic components.

degradation and solution of the pectic compounds in the tissues. Soluble pectin is developed in the juice during ripening at the expense of the insoluble pectose of the cell-wall, which exhibits a corresponding decrease in amount. As the fruit becomes soft and over-ripe, the pectin tends to undergo conversion into simpler decomposition products, and a temporary fall is observed in the pectin content of the expressed juice. Since the remainder of the pectose of the cell-wall continues to be progressively converted into pectin, no pronounced decrease is observed in the soluble pectin in the expressed juice until about May or June, when the greater part of the pectose has been decomposed. Meanwhile, from January onwards the pectic substances constituting the middle lamella begin to be decomposed and gradually pass into solution.

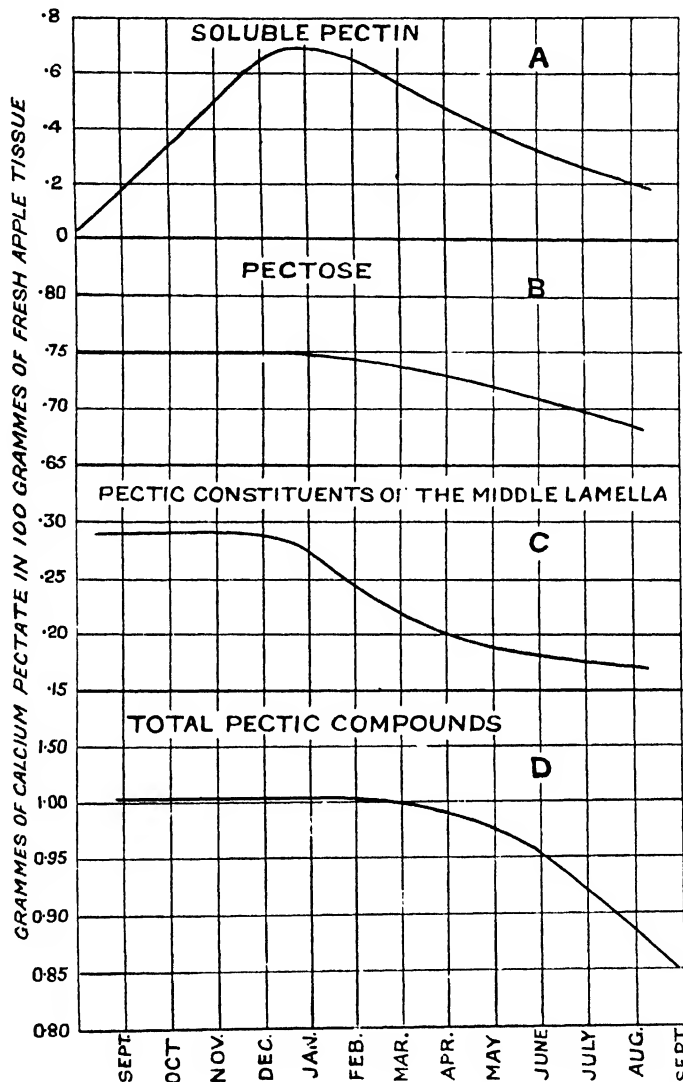
In the later stages of senescence (i.e. from about April onwards) there begins to be a marked diminution in the total pectic constituents. The pectose originally present has undergone decomposition, and only traces of the pectic substances of the middle lamella remain. The accompanying graph (Text-fig. 2) is a schematic representation of the average pectic changes in apples stored at 1° C.

III. HISTORICAL SUMMARY OF MICROSCOPICAL INVESTIGATIONS ON THE PECTIC SUBSTANCES.

The associations of the pectic substances with the cell-walls and middle lamella of plant tissues has long been recognized by a number of investigators. The most important of the early researches are due to Frémy (17-20), who investigated the structure and chemical composition of cells in considerable detail, demonstrating the pectic substances as fundamental cell constituents by effecting the preliminary removal of cellulose by Schweitzer's reagent, and identifying the residual cell framework with substances of pectic nature by chemical tests—such as solution by acids and alkalis. Frémy first pointed out the presence of an insoluble pectic compound—pectose—in fruits which gives rise on ripening to a water-soluble modification—pectin. Mulder (46) considered cells to be united by a kind of cell cement, 'inkrustierenden Substanz', which he showed to be of pectic nature, and regarded the cell-walls themselves as an intimate mixture of cellulose and pectose. Payen (49), Harting (26), Kabsch (34), Vogl (60), and later, Wiesner (65) and Tschirch (55) advanced similar views that pectic substances were exclusively confined to the middle lamella regions of cell tissues, of which they were the main constituents in the form of potassium or calcium pectates.

During the years 1889-93, Mangin published his important series of researches on the detection of pectic substances and their distribution in the soft tissues of numerous plants. Mangin accepts the hypothesis put forward

by Frémy, that the production of pectin is an important factor in the ripening of fruit, and considers that the development of the fruit is asso-



TEXT-FIG. 2. Graphical representation of the pectic changes in apples from the time of picking to the last stages of senescence.¹

ciated with fundamental changes in the pectic constituents. Mangin makes some attempt to locate the different pectic structures in various plants, basing his investigations on a combination of two methods: (1) the use of

¹ The chemical data are not complete, since they were only begun after the apple had been picked (i. e. after the period of full development), whereas the microscopical features were followed from the flowering period onwards; see Tables I and II.

basic stains such as safranin, methylene blue, and in his later work, ruthenium red, in order to differentiate the pectic compounds from the other constituents of the cell-walls; (2) the application of chemical reagents (alcoholic hydrochloric acid followed by alkali, ammonium oxalate, Schweitzer's reagent, &c.) to confirm the results obtained by staining methods. He concluded from such experimental evidence that pectose is intimately associated with cellulose in the substance of the cell-walls, and cannot be separated from it in an unchanged condition. He found that the lining of the air-spaces and the middle lamella were composed of pectic material which he regards as the calcium salts of pectic acid.

The results obtained by Mangin on the distribution of pectic substances in specialized plant structures were subsequently confirmed by a number of investigators. Among these may be mentioned the following: Vidal (59), who detected pectic substances in the roots of *Equisetum*, and Bancroft (24) who modified Mangin's methods and examined the pectic constituents in the xylem tissue of the *Pteridophyta*. Tunman (56) examined the roots of various Umbelliferae, and attempted to investigate the pectic metamorphoses of fruits by a preliminary treatment of sections with methylene blue, followed by immersion in sugar solution (a method suggested by Tschirch for the solution and separation of modified from unchanged pectic substances). Sampson (51) found that a series of pectic changes accompanied abscission in the leaves of *Coleus Blumei*. Howe (30) detected pectic material in the outer cell layers of root hairs, and Tupper-Carey and Priestley (57) investigated the distribution of pectic compounds in the apical meristem tissue of various stems and roots.

The origin and nature of the middle lamella has been investigated in considerable detail by Gardiner (21), and important contributions were made by Allen (1) and Devaux (14). Gardiner (21) describes the partial solution of the walls in the endosperm of *Tamus communis* during germination preceded by solution of the middle lamella. Allen (1) suggested that the middle lamella is of pectic origin, arising from secondary products which are inserted between the separated lamellae of the primary cell-wall. He regarded the middle lamella, not so much as a definite cell cement, but as a layer of plastic cell-wall material capable of undergoing rapid modification, and adapting itself readily to the particular requirements of the tissues.

Devaux (14) disputed the view that the composition of the middle lamella is exclusively calcium pectate, but inclined to the view that the pectic substances of plant tissues are all closely akin to pectose, being different members of a group of wall constituents. He agreed that calcium exists in the middle lamella, but pointed out the lack of evidence that it is in combination with pectic acid, or indeed that pectic acid exists as such at all in the middle lamella.

Hence there are varying opinions as to whether pectose or pectic acid constitutes the middle lamella of plant tissues. In the opinion of the writers, based on their investigations, it is unlikely that the middle lamella is composed of either pectose or pectic acid, but of a far more complex pectic substance, possibly containing residues of both pectose and pectic acid.

The significance of the pectic compounds in relation to bacterial and fungal attack, especially in connexion with the retting of flax, &c., has also been submitted to detailed investigation in the past. Van Tieghem (53) first showed definitely that bacterial agents were responsible for natural retting of textiles. He attributes the process to *Bacillus amylobacter* (*Clostridium butyricum*) an anaerobic organism which becomes active when rotting ensues in plant tissues, and whose prolonged action results in the isolation of the cellulose fibres. Later, Winogradsky (64), in conjunction with Friebes, pointed out that the retting of textile fibres was due to decomposition of the pectic cell cement by bacteria, and attributes this so-called 'pectic fermentation' to enzymes secreted by the bacteria themselves. Behrens (4) found that *Clostridium* was the chief agent in commercial retting and that numerous bacteria could effect it. He found that no action occurred on the cellulose, and compares the process of pectic disturbance to the changes induced by the chemical agencies employed by Mangin.

Meanwhile the relationship of pectic substances to fungal attack had received much attention. De Bary (3) observed that the fungus *Peziza sclerotiorum* caused the death of plant tissues by secreting a substance which effected partial disintegration of the cell-walls and which he attributes to an enzyme in the digestive juices of the invading fungus. Extracts of the diseased tissue caused a similar disintegration of the healthy tissue of carrots, but if previously boiled before inoculation no effect was produced. Marshall Ward (61) observed that various species of *Botrytis* have a similar effect on healthy plant tissue. Harding and Morse (25) and Jones (32), in a series of investigations on the soft rot of carrots and other vegetables, found that the attack of the bacillus involved solution of the middle lamella with the consequent separation of the cells in the areas of the invaded tissue. From an examination of the numerous bacteria producing soft rots, Jones concludes that a cytolytic enzyme (pectinase) is produced, capable of causing middle lamella solution. Cooley (13) and Hawkins (24), however, found that fungal disease does not invariably involve middle lamella solution, since the fungus *Sclerotinia cinerea*, (Bon.) Schroter, causing brown rots of plums and peaches, does not invade the middle lamella region of the tissues and does not apparently make use of pectin. Brown (8) obtained a powerful enzyme extract from the germ tubes of *Botrytis cinerea* and found that the action of this extract on various plant tissues (potato, turnip, beet, apple, &c.) operated in three stages, according to the time of action: (1) solution of the middle lamella, (2) *partial* disintegration of the cell-

walls, (3) death of the cells. In no case did Brown observe complete solution of the cell-wall, which observation is readily interpreted by assuming that the enzyme is only capable of acting on the pectic compounds of the cell-wall and middle lamella, and not on the cellulose components of the tissues. Confirmation of Brown's work is afforded by Valteau (58) in a series of investigations on the mode of attack of *Sclerotinia cinerea*. Valteau also attributes the solvent action to the secretion of an enzyme—the cytase or pectinase of other workers—and shows by a series of photomicrographs that the enzyme is secreted by the fungal hyphae in advance of the invading fungus. Willaman (62), also working on *Sclerotinia cinerea*, showed that if the fungus were grown on pectin as its sole source of nutriment, the pectin was converted into an insoluble gel of pectic acid, and that, ultimately, reducing sugars were split off from the pectic acid and assimilated by the fungus. Harter and Weimer (63) have obtained similar results with different species of *Rhizopus* and *Botrytis cinerea*, and find that the maximum macerating effect is observed when the capacity for acid production by the organism attains a pH value between 3.0 and 4.0.

None of these microscopical studies of the pectic substances, however, deal with their possible significance in the various phases of plant life—such as growth and development, the ripening of fruits, and the various stages accompanying senescence of plant tissues.

IV. MICROSCOPICAL METHODS FOR THE DETECTION OF THE PECTIC SUBSTANCES.

(a) *Application of Staining Reagents.* The basic stains, such as naphthalene blue, methylene blue, and safranin, recommended by previous investigators give unreliable results, since they do not exhibit specific affinity for pectic substances. On the other hand ruthenium red, a compound investigated by Joly (31) and employed with success by Mangin (45) for pectic mucilages, has proved entirely satisfactory. It does not stain pure cellulose, and possesses more specific affinity for pectic substances than any other stain used in botanical microtechnique. For these reasons ruthenium red has been employed in the course of this investigation for detecting the presence of pectic substances in plant tissues.¹

Gums, mucilages (45, 56), fatty acids (57), and gelose (56) have been recorded also as staining with ruthenium red, but the presence of these substances has not been detected in apples, and in tissues where they do occur

¹ The vascular tissue in apples is found to stain very strongly with ruthenium red, but it is probable that the staining is due to adsorption phenomena and does not necessarily denote the presence of pectic compounds, since it is unaffected by the application of the usual solvents for pectic substances. Also the vascular tissues in apple residues from which all substances of pectic nature have been previously removed, are observed to stain as intensely as before such treatment.

the pectic substances are readily distinguishable from them by characteristic chemical reactions (see Section VI).

In all cases freshly prepared hand sections were used for the tests, and serial sections were cut from a radial cylinder of the tissue in order to take into account variation in the individual apple. The variation in different samples consisting of ten individuals each of the same variety of apple was also observed.

(b) *Application of Various Pectic Solvents.* It has been found possible to recognize the structures in the apple tissue which stain with ruthenium red as substances of a pectic nature by the application of chemical reagents known to effect the removal of the pectic constituents of the tissues (see Section II). Ammonium oxalate is useful for this purpose, since it readily dissolves out all the pectic compounds, leaving the cellulose unaltered. Careful treatment of the sections with hydrochloric acid followed by potassium hydroxide also effects the removal of these compounds, the use of these reagents being an adaptation of the chemical methods already described. The degree of alteration induced by this treatment depends upon the concentration and the time of action of the reagents. Prolonged action results in the more or less complete removal of the pectic compounds, and it was observed that the cell-walls no longer stain with ruthenium red and that the cells become separated from one another owing to the solution of the middle lamella.

(c) *Application of Cellulose Solvents.* The use of the purely chemical and microscopical methods described above confirms the view that the cellulose of the cell-walls is intimately associated with pectose, and that the middle lamella may be conceived as a kind of cell cement, composed of a complex containing pectic acid or pectates, which encases and binds together the component cells. This conception of the distribution of the pectic compounds in the tissues is supported by the evidence obtained from the use of the reverse process of dissolving out the cellulose and examining the disposition of the remaining cell-wall substances. Sections were treated with Schweitzer's reagent, according to a method first described by Frémy (17-20) and later developed by Mangin (38-45), which removes the cellulose and leaves the pectic substances in an insoluble condition in the tissues, thereby maintaining the original framework. After this treatment the sections are extremely fragile, but microscopical examination shows that the structural appearance of the tissues is unaltered, the outline of the cells being maintained by the presence of the pectic constituents. Ruthenium red stains this residual framework deeply, and subsequent treatment with pectic solvents (ammonium oxalate, or hydrochloric acid followed by weak alkalis) gradually dissolves away the framework, leaving no visible sign of the original structures.

The authors have found that a combination of staining methods and

the application of chemical reagents is entirely satisfactory for the examination of the disposition of the pectic substances in plant tissues and of the changes which they undergo, and have adopted these methods consistently throughout this investigation.

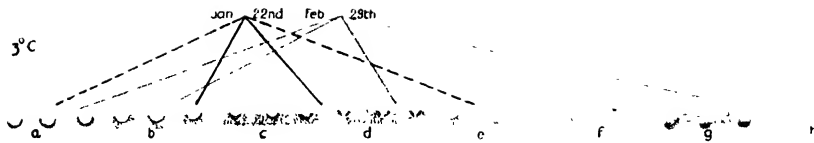
V. PECTIC CHANGES DURING THE GROWTH AND SENESCENCE OF NORMAL APPLES AND PEARS AS OBSERVED BY MICROSCOPICAL METHODS.

(a) *From the Flowering Period onwards until the Apple has attained its Maximum Size.*

In the young fruit the tissue is compact, the parenchymatous cells exhibit regular outlines, and the intercellular spaces are exceedingly minute (Pl. XII, Fig. 1). As development proceeds, the cells become irregular in shape and size, the cell-walls become thinner, and the intercellular spaces are enlarged. At first the cell-walls are uniformly stained with ruthenium red, indicating the even distribution of pectic substances throughout the walls, but at quite an early date (June) the walls begin to exhibit unequal staining (Pl. XII, Figs. 2-4), the portions of the wall abutting on the intercellular spaces being more strongly stained than those in contact with other cells. For convenience in description, the portions of the wall of a given cell *in contact with* and *free from* respectively another cell will be termed 'walls of contact' and 'free walls' in the course of this paper. As ripening proceeds, more pronounced changes are evident, of which full details are given in chronological order in Table I.

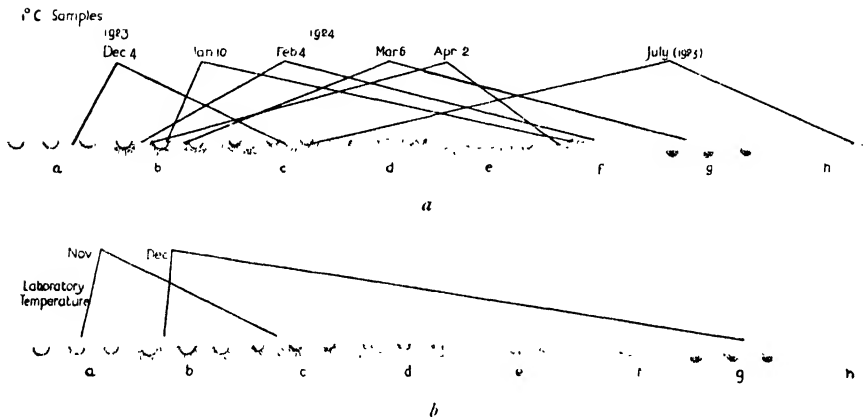
At the close of this developmental stage the middle lamella consists of an extremely thin layer of pectic material, which is not easily distinguishable from the remaining cell-wall substance. The disposition and the forms assumed by the other pectic constituents of the cell-wall (Pl. XII, Fig. 5) are as follows :

(1) *Discs*. These structures are extremely small aggregations of pectic material formed during cell-growth. They vary in number and size ($2-8\ \mu$), and are distributed irregularly in the walls of contact. In surface view they present a more or less elliptical or circular form, and sometimes the outline is irregular. When fully developed they are not uniformly stained, the surface exhibiting a finely granular or alveolar appearance. In sectional view they are observed as short rods of stainable material, and the discs situated in the wall of contact of a particular cell stand opposite those present in the neighbouring wall of contact of the cell to which it is attached. (2) *Crescents*. Aggregations of pectic substance staining deeply with ruthenium red. These structures are usually larger than the discs and are disposed as a rule near the boundary of a wall of contact. In surface

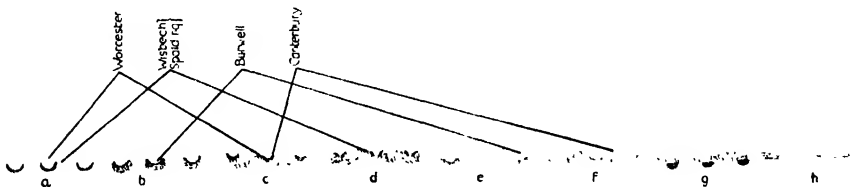


TEXT-FIG. 3. Variation in degree of change of crescents occurring among the individuals in samples of Bramley's Seedling apples stored at $1^{\circ}\text{C}.$, and examined at different dates, i. e. January 22 and February 29.

Jan. 22 ——— minimum degree of variation in sample.
 " ——— maximum " " " " "
 Feb. 29 ——— minimum " " " " "
 " ——— maximum " " " " "



TEXT-FIG. 4 *a* and *b*. Variation in the degree of change of crescents exhibited by different samples of Bramley's Seedling apples during senescence. 4 *a*. $1^{\circ}\text{C}.$ 4 *b*. Laboratory temperature.



TEXT-FIG. 5. Variation in the degree of change in the crescents exhibited by samples of Bramley's Seedling apples from different localities examined at the same time.

view they present a crescentic form with the horns of the crescent directed towards the centre of the wall. The crescents do not exhibit any obvious structural details. In a sectional view their appearance varies with the

TABLE I.
*Details of the Pectic Changes observed during the Development
of the Bramley's Seedling Apple in 1924.*

<i>Date.</i>	<i>Dimensions of the Apple in cm.</i>	<i>Dimensions of the Cells in μ^1.</i>	<i>Dimensions of Air- spaces in μ^1.</i>	<i>Microscopical and Chemical Observations.</i>
May 27	Diam. Ht. 0.9 x 1.1	38 x 38	36 x 12	Tissues compact, numerous thin partition walls present.
June 12	1.8 x 1.5	90 x 67	114 x 36	Cell-walls show signs of unequal staining.
June 24	3.5 x 3.0	118 x 85	203 x 107	Unequal staining more pronounced. The stained portions finely granular.
July 2	4.3 x 5.5	227 x 136	213 x 80	Minute feebly stained areas (<i>discs</i>) ³ present in the walls of contact. A little starch present.
July 16	6.0 x 5.0	186 x 135	366 x 135	The walls of contact are bounded by a narrow band of pectic substance. Much starch. Parchment layer lining the ovarian cavity stains with phloroglucin.
July 25	6.5 x 4.7	—	—	<i>Bands</i> ³ more prominent up to 8 μ wide.
Aug. 8 ²	6.7 x 6.0	—	—	Minute extracellular projections (<i>papillae</i>) ³ observed. Traces of pectin appear in the expressed juice.
Aug. 14	7.0 x 5.5	—	—	<i>Papillae</i> ³ more numerous. Traces of pectin appear in the expressed juice.
Sept. 3	8.0 x 5.5	—	—	<i>Bands</i> ³ less prominent. Small crescentic bodies (<i>crescents</i>) ³ present in the bands, but of rare occurrence. Slight increase in pectin in the expressed juice.
Sept. 17	7.0 x 6.0	—	—	Starch feebly developed. A) appreciable amounts of pectin present in the expressed juice.
Oct. 25	8.5 x 6.0	248 x 190.4	—	<i>Bands</i> ³ feebly developed. <i>Crescents</i> ³ and <i>discs</i> ³ distinct and fairly numerous. Starch absent.

particular angle of inclination of the wall under observation. (3) *Bands*. Thin bands of pectic substance which occur at the boundaries of walls of contact. At maturity, if present, the bands are usually only feebly stained with ruthenium red. (4) *Papillae and small globules*. Minute extracellular projections with which the free walls of the cells are studded. These

¹ In arriving at these dimensions the average of not less than twenty of the largest of the cells or intercellular spaces observed in one or more sections is given in each case.

² Systematic chemical investigations were begun about this date.

³ For convenience in description the pectic structures described above will be referred to respectively as discs, bands, papillae, and crescents, in the course of this communication.

structures stain feebly with ruthenium red. The papillae may be regarded as initial stages in the formation of the small globules, which are developed in great abundance during the late stages of senescence.

The observations recorded in Table I indicate that during the development of the cells and intercellular spaces the pectic substances undergo a series of gradual changes. The discs, crescents, and extracellular projections do not develop simultaneously, and the appearance of the crescents is preceded by the peculiar bands of pectic substances already described. These bands are often absent when the apples are fully matured and the crescents are well developed, and it will be shown subsequently that they are again manifest when the crescents are undergoing further changes during the period of senescence.

The varieties Bramley's Seedling, Cox's Orange Pippin, and Worcester Pearmain exhibit similar pectic changes during the development of the apple. With the Worcester Pearmain apples the development of the various pectic substances takes place somewhat earlier than with the Bramley's Seedling; this difference is probably related to the characteristics of the classes of apples which these varieties represent. The Worcester Pearmain ripens relatively early, but does not keep well. Bramley's Seedling, on the other hand, is a late variety and possesses good keeping qualities.

Since the cell-wall is extremely thin, it has not been possible to determine accurately by the technique employed in this investigation whether the crescents and discs are situated in the inner layer of the wall or middle lamella region. It is regarded as probable that they bear some relation to the pectic changes occurring in both the inner and outer wall regions.

In attempting an interpretation of the appearance and changes undergone by the pectic substances described above, it may be suggested that they are due to the expansion of the cells. During the building up and growth of the cell-wall, the uniform layer of pectic material which was primarily laid down in the young cell-wall must become spread over a larger area as the cells enlarge, since preliminary chemical analysis shows that there is no appreciable increase in the amount of pectic material in the cell-wall during this period of growth. The pectic constituents thus form an increasingly thinner layer, which may be conceived to give rise either to the disconnected areas seen in the adult tissue as unevenly staining regions in the cell-wall, or possibly contribute to the disc-like structures which occur in the middle lamella region of walls which are in contact with one another.

It is important also to note in interpretation of the facts observed that throughout this period the development of the intercellular spaces involves modification of the middle lamella, which is due to the partial mechanical

separation of the cells. During the primary development of the air-spaces globules are not observed, but it will be shown subsequently that they appear later and are associated with secondary modifications of the inter-cellular spaces.

It may be concluded from these observations on the redistribution of the pectic substances during growth, that they constitute a plastic layer capable of considerable modification and adaptation as development of the tissues proceeds.

(b) *From the Preceding Stage until the Onset of Physiological Break-down.*

This stage has been studied chiefly in apples kept under low temperature conditions. It is marked by a slow modification in form of the various structures constituting the pectic framework, culminating, when physiological break-down ensues, in their complete disappearance. Stability to pectic reagents decreases gradually throughout this stage of development, the pectic structures being readily brought into solution.

Before embarking on this study of changes during senescence, the question of the variability of the pectic substances, that is to say, variation in time of the appearance of different stages of pectic transformation, was studied in considerable detail. The probable range of the variation in the individual apple was thus ascertained. A certain amount of variation was found on comparing sections taken from the inner and outer regions of the flesh, from different sides, and from the upper and lower portions of the individual apple. The variation in degree of pectic change shown by an individual apple is represented diagrammatically in Text-fig. 3 (facing p. 210), where the letters *a-h* denote different stages in the transformation of the crescentic bodies (see Pl. XII, Figs. 6-14). The examination of individual apples during senescence shows that the stability of the pectic substances (papillae and small globules excepted) is least affected in the inner regions of the tissues; they are, in fact, observed to become progressively more unstable in a radial direction outwards, the maximum change being detected in the periphery. This indication of more advanced change in the pectic constituents situated in the peripheral flesh of the apple may be readily attributed to the fact that this tissue region is nearer the external air than the more deeply seated tissue, and would be directly affected by factors (light, temperature, &c.) which affect the process of ripening.

The variation existing in a sample consisting of a number of apples was also ascertained. Generally speaking, the least variation is found at the commencement of the period of senescence. The crescents and discs are more or less uniformly distributed, and usually well developed, while the middle lamella as yet shows no marked signs of alteration.

During senescence variation increases, and the difference in the degree of pectic change observed when different parts of the same apple or different apples from an average sample are compared becomes more and more evident.

Owing to this variability in different parts of the same apple and in different apples, the method of investigating pectic changes was standardized. Radial cylinders of tissue were taken by means of a cork borer from different sides of the apple. From each cylinder several tangential sections were made at each of three different positions chosen at distances of approximately 0.5, 1.5, and 2.5 cm. respectively from the skin. This process was carried out on representative samples of apples obtained at regular intervals from the Low Temperature Research Station at Cambridge.

The changes which the pectic substances undergo during the period of full maturity and senescence may be outlined briefly as follows:

(a) The discs (Pl. XII, Figs. 6-14) exhibit structural alteration and become swollen (Pl. XII, Figs. 9-11). Later, they show a marked diminution in their capacity for staining with ruthenium red (Pl. XII, Fig. 12), and finally disappear (Pl. XII, Figs. 13 and 14).

(b) The crescents (Pl. XII, Figs. 6-14) also undergo a definite sequence of changes. At first they become surrounded with diffuse stainable material (Pl. XII, Fig. 7). Later the stainable material surrounding neighbouring crescents coalesces, forming a continuous band (Pl. XII, Figs. 8 and 9), which follows the boundary of a wall of contact. Meanwhile the crescents appear to be losing substance (Pl. XII, Fig. 9), and when the bands are prominently developed, only the outlines of the crescents remain (Pl. XII, Figs. 10 and 11). Finally, when the areas undergoing solution abut on an intercellular space, large globules are formed which project from the bands into the intercellular space (Pl. XII, Figs. 10 and 11). In the later stages complete disappearance of the bands and globules is observed (Pl. XII, Figs. 13 and 14).

These changes in both crescents and discs bear the interpretation that pectose is steadily breaking down to produce soluble derivatives—pectinic acids and ultimately pectin. Pectin in its turn is gradually decomposed into other soluble bodies, sugar, organic acids, &c., which no longer exhibit the typical staining reactions of the pectic complex. This progressive decomposition accounts for the altered appearance of the discs and crescents and for the diffuse pectic material which surrounds the latter. These structures may be supposed to absorb water from the intercellular passages, and possibly from the cell contents,¹ thereby producing the phenomena of swelling, gradual solution, and finally globule formation as described above.

¹ Decomposition of the pectic constituents of the cell-wall will tend to facilitate the outward passage of water.

The fact that it is possible to reproduce such phenomena artificially, and that ready solution of the diffuse material referred to can be brought about by irrigation or soaking sections in water, supports the above-mentioned interpretation of the changes under discussion. It may be pointed out also that the pectose in the cell-wall is much less stable towards reagents at this stage than it is in the earlier stages of development.

(c) The papillae and small globules increase in size and number during this phase, and are more readily stained with ruthenium red than before (Pl. XII, Fig. 5).

The development of these bodies may be attributed also to the steady production of pectin and its tendency to absorb and dissolve in any water which may be in contact with it. The increase in staining capacity of the globules would necessarily follow from increase in the size and concentration of their substance as the process of pectose conversion continues.

(d) The cells tend to separate from one another, assuming a bottle-like form, and minute aggregations of pectic material are left in the separated walls (Pl. XIII, Fig. 24, and Pl. XIV, Fig. 25). This suggests that decomposition of the middle lamella pectic substances has now set in, resulting in the initial stages of cell separation.

The period of full ripeness is characterized by further microscopical changes. The various pectic structures associated with the cell-wall become diminished in size or disappear altogether, the cell-walls appear thin and transparent, and the tissues stain very feebly as a result of the decomposition of their pectic components (see Pl. XII, Fig. 14). The middle lamella undergoes further decomposition, resulting in a soft condition of the apple, as the cells tend to separate from one another. In over-ripe fruit and in the last stages of senescence, these features become more pronounced. The cells separate from one another with slight mechanical pressure, or on immersing the sections in water. The spaces between the separated cells are often occupied by a stainable matter of stringy consistence, presumably consisting of masses of semi-soluble pectic material derived from the disintegrated cell-walls and middle lamella (Pl. XIV, Fig. 32). The majority of the separated cells are dead, as judged by their plasmolysed appearance and altered refractive index (Pl. XIII, Fig. 24, and Pl. XIV, Fig. 25). The cell-wall appears thin and wrinkled, and the protoplasm exhibits a yellow colour.

The accompanying table (Table II) gives a summary of the pectic changes which were recorded during the ripening and senescence of Bramley's Seedling apples stored at 1° C. for a period of eight months.

With reference to Table II the following points are of special interest : (1) During the period from October until the beginning of December, when the first records appearing in Table II were made, the pectic structures in the tissues exhibited little change. Owing to the incidence of 'internal break-

down' in the store from April onwards, the number of apples available for microscopical study was very limited, and it is therefore doubtful whether the surviving samples actually show normal senescent changes. In the previous year (1922-3) 'internal break-down' was less prevalent in Bramley's Seedling apples held under similar storage conditions, and the final stages of normal senescence were reached in average samples in July (1923). Accordingly, the observations made in July, 1923, are given in addition to those of the following year. In 1923 the final stage of senescence in average samples was marked by the concurrent dissolution of the various pectic constituents (globules, cell-wall pectic substances, middle lamella, &c.). The apples exhibited the condition known to the trade as 'mealiness', and the constituent cells of the sections were readily separated by applying slight pressure. A little later, sections showed more or less complete separation of the cells, and the loosened cells exhibited the characteristic bottle-like shapes. In a large number of cases marked plasmolysis of the cell contents was observed also.

In 1924, however, the fruit surviving 'internal break-down' appeared to be more resistant to physiological break-down than the average samples examined in 1923. A general diminution of the pectic substances associated with the cell-walls had taken place before solution of the middle lamella became pronounced, and the condition of 'mealiness' was less evident. Nevertheless, by gentle pressure the cells were readily forced apart, and by careful manipulation with needles it was found possible to change the shape of the individual cells, and to draw them out into the bottle-shaped forms characteristic of the 1923 samples. It seems likely, therefore, that if the stock of apples in 1924 had not become thus depleted, the average samples would have exhibited the phenomena recorded in 1923.

(2) An inspection of the table shows that the factors operating during senescence do not produce simultaneously the same degree of change in the various pectic substances. For example, at a time when the crescents had undergone considerable modification (January 10), the cohesion of the cells was not affected to any marked extent. A similar lack of uniformity in behaviour at a given time was noted in the changes affecting the crescents and discs and papillae respectively. Results obtained from the purely chemical side of this investigation suggest a feasible explanation of these observed facts, in that the various bodies show constitutional variation which is obviously correlated to differences in their susceptibility to the chemical changes obtaining in the maturing fruit.

It will be of interest to compare the changes recorded in Table II with the pectic changes as observed by chemical methods carried out concurrently on similar samples of apples. Chemical methods show that during ripening the tissues gradually become depleted of pectose as it is converted

into the form of soluble pectin. The period of full ripeness is characterized by changes in all three of the pectic constituents, pectose, pectin, and the middle lamella pectic substances. The rate of pectose decomposition is

TABLE II.

Pectic Changes observed in Bramley's Seedling during Storage at 1° C.

<i>Date.</i>	<i>Crescents.</i>	<i>Discs.</i>	<i>Papillae and Small Globules.</i>	<i>Middle Lamella Pectic Substances.</i>	<i>Cell, Shape, Staining, &c.</i>
1923. Dec. 4	<i>Crescents</i> little altered, <i>bands</i> feebly developed, <i>large globules</i> absent.	Not prominent, distinct on boiling (H ₂ O).	None observed.	Unchanged.	Normal.
1924. Jan. 10	<i>Crescents</i> swollen, <i>bands</i> present, occasional <i>large globules</i> (peripheral flesh).	Not prominent, distinct on boiling (H ₂ O).	<i>Papillae</i> not prominent.	Swollen pectic substance at the air-space angles.	Normal.
Feb. 8	<i>Crescents</i> altered, <i>bands</i> prominent, <i>large globules</i> present (peripheral flesh).	Slight development.	<i>Papillae</i> and <i>small globules</i> moderately prominent.	Swollen pectic substance at the air-space angles.	Occasional <i>bottle-shaped cells</i> .
Mar. 6	The changes have extended to the central regions of the flesh.	Prominent.	Very <i>small globules</i> present.	Cells show signs of separation.	Occasional <i>bottle-shaped cells</i> .
Mar. 15	Slight extension of changes.	Prominent.	<i>Small globules</i> prominent and numerous.	No further change.	Occasional <i>bottle-shaped cells</i> .
Apr. 2	Slight extension of changes.	Altered.	No further change.	No further change.	Occasional <i>bottle-shaped cells</i> .
May 15	Remains of <i>crescents</i> and <i>bands</i> , <i>large globules</i> numerous.	Much altered or absent.	No further change.	Pectic substances less prominent.	Cell-walls not well stained.
Aug. 6	Remains of <i>crescents</i> and <i>bands</i> and <i>large globules</i> .	Traces only.	In stages of disappearance.	Cells separating.	<i>Bottle-shaped cells</i> common, cell-walls show general lack of pectic substance.
1923. July 3	Remains of <i>crescents</i> , <i>bands</i> , and <i>large globules</i> .	Much altered or absent.	In stages of disappearance.	Cells separating, with strands of pectic substance between cells.	<i>Bottle-shaped cells</i> prevalent and cell-walls stain feebly and in patches (irregular areas).

accelerated, resulting in an accumulation of soluble pectin in the tissues. As the fruit becomes over-ripe the pectin breaks down further into soluble decomposition products and a decrease is detected then in the middle lamella pectic substances as they also pass into solution. This process of degradation continues gradually, a marked decrease in total pectic constituents being

observed during the last stages of senescence, and the pectin and middle lamella pectic substances disappear completely in extreme cases (see Text-fig. 2).

It may be concluded, therefore, from the investigations on both the chemical and microscopical sides, that the series of progressive changes in the pectic compounds which are distributed in the cell framework are intimately associated with the gradual metabolic drift in the apple from maturity to the final stages of senescence. It may be stated, in fact, with a considerable degree of assurance, that certain pectic changes are characteristic of the particular stage of maturity or senescence which a sample of fruit under examination has reached, and that the converse case may be usefully applied to the commercial aspect of the problem, namely, that the stage of development or maturity of a given sample of apples can be gauged approximately by either a microscopical or a chemical examination of the tissues.

At the present stage of this work it is hardly possible to form a satisfactory conception of the mechanisms promoting pectic disturbances. Judging from the results obtained by the use of reagents on sections of tissue (see Section VI), the pectic decompositions appear to be a series of hydrolytic changes. The possibility of enzyme activity in the regulation of such changes is, of course, obvious, any such activity being regulated by modifications in the cell contents.

(c) *Effect on Changes in Bramley's Seedling Apples of Storage at Various Temperatures.*

No acceleration of the pectic changes was observed as a result of storing at a slightly higher temperature, i.e. 3°C . (Pl. XIII, Figs. 20-3), but a considerable rise in temperature, for instance to 15° , or storage under ordinary laboratory conditions, resulted in acceleration of the pectic changes (Pl. XIII, Figs. 18, 19). Thus the degree of pectic change reached by apples stored at 1°C . in March was comparable with that reached about three months earlier by apples stored under laboratory conditions. In Text-fig. 4 the differences in behaviour of the pectic substances at 3°C . and laboratory temperature is presented diagrammatically. These observations are in agreement with those made as the result of chemical estimations of the pectic changes in similar apples held at laboratory temperatures and at 1°C . respectively (10, 12).

(d) *Variation in Changes in Bramley's Seedling Apples obtained from Various Localities.*

It was observed that the same variety of apple obtained from different localities and examined at the same time (November, 1923) exhibited varying degrees of pectic change. Thus, apples from Burwell (fen-land) and

Canterbury (gravel) showed more pronounced changes than those exhibited by apples from Spalding (silt), Wisbech (fen-land), and Worcester (Old Red Sandstone). Text-fig. 5 illustrates the variation in the degree of change exhibited by the crescents (as representative of the general trend of pectic changes) in apples obtained from five localities and examined at the same time.

(e) *Variation in Changes due to Variety of Apple.*

Generally speaking, the various phenomena exhibited by pectic substances in different varieties of apple do not differ markedly from those recorded for Bramley's Seedling. Differences in detail are found, for example, in size and number of the crescents, size and degree of distinctness of the discs, degree of prominence of the papillae, globules, &c. But in all the varieties examined the same type of change was observed, e.g. crescent alteration, development of small globules during late senescence, development of bottle-shaped cells, and progressive solution of the middle lamella pectic substances.

Differences in the rate of change were observed for different varieties which appear to be related to the maturity and keeping properties of the apple. Thus in the case of a rapidly maturing variety which is in season for only a short period, the sequence of changes is observed to take place much more rapidly than with Bramley's Seedling, a variety recognized as having good 'keeping' properties.

Among the varieties specially examined and compared were Bramley's Seedling, Bismarck (Pl. XIV, Fig. 20), Cox's Orange Pippin, Lane's Prince Albert, Newton Wonder, Allington Pippin, and Worcester Pearmain. Several of the more important varieties imported from the Dominions were examined also.

(f) *Pectic Changes in the Pear* (Pl. XIV, Figs. 47-51).

Preliminary observations made on the pectic substances present in pear tissues, during the period of storage at low temperatures, from the autumn until March, show that they differ in several respects from those of the apple, both in the forms which they assume and their behaviour during senescence. Thus, during the period of development the crescents which are characteristic of the apple are less frequently observed, and when present are much less conspicuous. For this reason the transformations which these structures undergo during senescence in the apple were not apparent. The development of numerous small globules in the free walls has not been observed. As with the apple, the cell-walls are not uniformly stained, but this feature is sometimes less prominent in the pear. On the other hand, the discs are numerous, but small. Pears in various stages of decay were

examined in March. The chief features observed were (see Pl. XIV, Figs. 47-51):

(1) Discs swollen and globular in form; (2) surface of the free walls, when stained with ruthenium red, exhibited a granular appearance; (3) the various manifestations of pectic change associated with the final dissociation of apple tissues were not observed, namely, the occurrence of irregular patches of pectic materials in the cell-walls, the extensive development of globules, and the presence of masses and strands of pectic materials between the cells; (4) bottle-shaped cells were prevalent.

In the case of apples in the condition of natural decay, the tissues readily dissociate when placed in water, or do so when gentle pressure is applied. This is not the case, however, with pear tissue; indeed, the cells often remain attached after the application of considerable pressure. This persistence in the cohesion of the cells appears, however, to be due to the presence of groups of stone-cells, which resist mechanical pressure and prevent its application to the parenchymatous cells, rather than to the presence of pectic substances acting as cell cement. This view is supported by the fact that if the stone-cells are first removed it is then possible to bring about the separation of the parenchymatous cells. Hence, although the microscopical examination of the pear reveals very few of the pectic phenomena common in the apple, the net result is the same in both fruits, involving more or less complete dissolution of the pectic framework accompanied by separation of the cells and plasmolysis of the cell contents. Since the processes of development, maturation, and senescence as a whole occupy a markedly shorter period of time in the pear as compared with the apple, it may be suggested that the lack of pectic structures is due to the temporary nature of their existence in a fruit which is undergoing more rapid metabolic changes.

The results obtained in this case of the pear¹ by adopting standard methods for extracting and estimating the various pectic constituents suggests that these constituents do not differ essentially, either in actual percentage content or in chemical constitution, from those present in apple tissues. This conclusion is borne out by the authors as a consequence of the results obtained by microchemical examination of pear tissue. Detailed comparisons of these fruits show that an important difference is a much lower acid content in pears as compared with most apples (Conference pear, 0.092 gm. malic acid per 100 gm. fresh weight; Bramley's Seedling 1.2 gm. malic acid per 100 gm. fresh weight of apple tissue).² It may be suggested, therefore, that acidity is the controlling factor in pectic metamorphoses, namely, in cell-wall degeneration, and that a low acid content involves a lessening of the protoplasmic control of the enzymes producing pectic

¹ Chemical investigations have been carried out by Miss A. M. Emmett, and the detailed results will shortly be published.

² See note, p. 213.

decomposition. It is interesting to note in this connexion that certain varieties of apples which have a low acidity (Cox's Orange Pippin, 0.666 grm. malic acid per 100 grm. of fresh weight, Beauty of Bath, 0.701 grm., and Worcester Pearmain, 0.191 grm.)¹ were proved to be those which very rapidly exhibit the tissue softening ('mealiness') which accompanies pectic decomposition.

VI. EFFECT OF HYDROLYTIC REAGENTS ON THE PECTIC CONSTITUENTS OF APPLE TISSUES (Pl. XIV, Figs. 26-8).

All the pectic transformations associated with the various phases of ripening and normal senescence of the apple fruit have been induced by treating sections with the appropriate solvents, viz. water, hydrochloric acid, potassium or sodium hydroxide, and ammonium oxalate, by carefully adjusting the temperature conditions and concentration of the reagents. Owing to the variation in the degree of pectic change occurring in individual apples, each experiment made in this connexion was repeated again and again until it appeared certain that the recorded changes were caused by the reagent employed. Two methods were adopted: (1) tangential sections were taken (using a radial cylinder of tissue) at a measured distance from the core, some of which were used for experimental purposes and the remainder kept as controls: (2) a particular section was chosen, and after recording the degree of change exhibited by the pectic structures, the section was then treated with the reagents and a second record made. On several occasions the same section was treated repeatedly either with the same or different reagents. This method was followed in all cases where inconclusive results had been obtained by the first method.

Sections soaked in water for short intervals of time (24 hours) gave variable results, but after more prolonged treatment, pronounced changes were evident, the crescents and discs had undergone alteration, and large globules were present. The changes observed in soaked sections were probably partly an effect of the lesions made in preparing the sections (see p. 223).

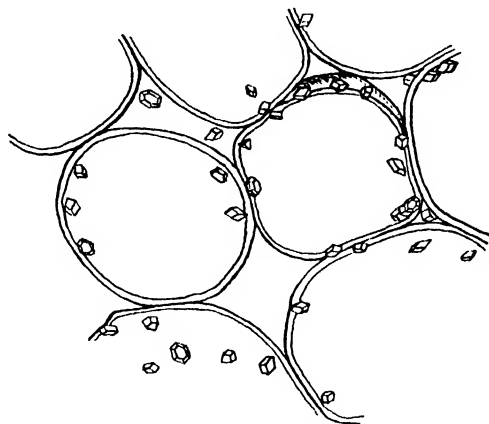
Boiling in water for periods of from 5 to 10 minutes induced considerable alteration in the pectic substances. In certain cases, by boiling sections in water and cooling rapidly, crystals were observed which were deposited round the cells following the lines of the middle lamella (Text-fig. 6). Further details are given in the accompanying table (Table III) of results from a representative series of experiments.

In Table III the dates of each experiment are given, since it is important to record the initial degree of development of the fruit before the experiment is begun, in order to arrive at a definite conception of the actual degree of alteration induced.

The results obtained from macrochemical investigations offer a ready

¹ These acid values were kindly furnished by Mr. D. I. Evans.

interpretation of the above phenomena (see Section II). Provided the sections are in a sufficiently immature condition, the treatment with boiling water



TEXT-FIG. 6. Crystalline deposit obtained by boiling sections in water and cooling rapidly.

TABLE III.

Illustrating the Effect of Boiling Water on the Pectic Substances present in the Tissues of Bramley's Seedling Apples.

<i>Origin of the Apples and Storage Temperature.</i>	<i>Date.</i>	<i>Condition of Pectic Substances before Treatment.</i>	<i>Treatment.</i>	<i>Condition after Treatment.</i>
Spalding (Silt), Lab. temp.	21. 11. 23	Crescents not observed.	Boiled 5 min. Reboiled 10 min.	Crescents numerous. Crescents less numerous and showing signs of change.
Burwell (Fen), Lab. temp.	23. 11. 23	Distinct crescents rare, bands feebly developed.	Boiled 5 min.	Few crescents, bands prominent.
Burwell (Fen), 1° C.	22. 1. 24	Crescents moderately abundant, some altered; bands narrow, discs distinct.	Soaked 24 hours, then boiled 5 min.	Crescents scarce, bands prominent, discs scarce.
Burwell (Fen), 3° C.	1. 3. 24	Crescents numerous, some showing signs of alteration. bands feebly developed, no large globules.	Boiled 5 min.	Crescents altered, large globules observed.
Worcester (Old Red Sandstone), 3° C.	10. 3. 24	Crescents numerous, some showing signs of alteration, no large globules.	Boiled 5 min.	Prominent bands, large globules observed.
Burwell (Fen), 1° C.	6. 3. 24	Crescents altered, bands prominent, discs indistinct, large globules numerous.	Soaked 18 hours, then boiled 5 min.	Large globules not observed.

may be supposed to hasten the production of pectin from the pectose in the tissues, and soluble pectic substances from the middle lamella complex, producing the usual transformations of the pectic structures. Treatment of

sections in a more advanced state of maturity produces the phenomena which are usually associated with the final stages of senescence, namely, the various substances are dispersed, their place being taken by large globules or diffuse stainable masses which after prolonged boiling completely dissolve and disappear.

TABLE IV.

Illustrating the Effect of HCl, KOH, and Ammonium Oxalate on the Pectic Substances present in the Tissues of Bramley's Seedling.

Condition of the Pectic Substances.

<i>Before Treatment.</i>	<i>Treatment.</i>	<i>After Treatment.</i>
(1) Crescents not observed. Bands not prominent. Discs numerous Large globules absent. Cell-walls well stained (21. 11. 23).	(i) Soaked M/10 HCl 12 hrs. (ii) Boiled M/10 HCl 10 min. (iii) Boiled M/10 HCl, then soaked M/10 KOH 24 hrs.	(i) Little change. (ii) Discs present. Cell-walls feebly stained. Tissue disintegrating. (iii) General loss of pectic substance. Discs very indistinct. Cell-walls stain in patches.
(2) Distinct crescents few. Bands feebly developed. Discs numerous, indistinct. Cell-walls well stained (23. 11. 23).	(i) Soaked 2N HCl 12-72 hrs. (ii) Soaked 2N HCl 12 hrs., then soaked NaOH 12 hrs.	(i) Remains of crescents. Bands more distinct. Cell-walls well stained. (ii) Outlines of crescents. Cell-walls stain in patches.
(3) Crescents moderately abundant and distinct. Bands present. Discs numerous and distinct. Cell-walls well stained (26. 11. 23).	(i) Boiled KOH.	(i) Crescents and discs absent. Cell-walls feebly stained. Tissue disintegrating.
(4) Apple exhibits advanced pectic changes (3. 7. 24).	Soaked 5 % HCl 3 days, then boiled 5 % KOH.	Pectic structures absent from cell-walls; lumps of pectic material scattered about the section.
(5) Crescents and altered crescents moderately abundant. Bands present. Large globules absent. Discs numerous, somewhat altered. Small globules present (7. 3. 24).	(i) Soaked H ₂ O (5 days). (ii) Soaked ammonium oxalate 5 days. (iii) Boiled ammonium oxalate.	(i) All pectic substances changed, much diffuse pectic substance present, and numerous large globules. (ii) Little pectic substance left, no large globules. (iii) Pectic substances absent; section disintegrating.

The effect of the water therefore appears to be a hydrolytic decomposition of the pectic substances already existing in a labile condition, followed by solution of the decomposition products.

Soaking in cold dilute HCl (M/100) failed to produce any marked pectic changes; the changes were more marked when the concentration was

considerably increased, but even then the cell-wall stained strongly with ruthenium red. Repeated boiling in dilute HCl (M/100) did not materially affect the pectic substances. More pronounced effects were obtained on increasing the concentration; nevertheless, when the operation of boiling was continued until the section disintegrated, pectic substance was still present in the cell-wall, and globules, when present, were strongly stained with ruthenium red. Treatment of sections with cold dilute acid, followed by immersion in cold dilute alkali for sufficiently prolonged periods, produced marked changes (Pl. XIV, Figs. 26 and 27). Compare also Pl. XIV, Fig. 29. Boiling in HCl, followed by KOH or NaOH, caused disintegration of the sections and an almost complete disappearance of all the pectic constituents of the cells (Pl. XIV, Fig. 28). Boiling ammonium oxalate also brings about the disintegration of all the pectic substances. Some typical examples of the experiments made with HCl, KOH, and ammonium oxalate are given in Table IV.

The following interpretation is given in explanation of the above observations: All the pectic substances (pectose, pectin, pectic acid, &c.) are practically insoluble in cold dilute hydrochloric acid, hence no pectic changes are apparent. Boiling with dilute HCl (M/100) causes hydrolysis of pectose with production of pectin, and probably a certain amount of pectin break-down products of acid nature will be formed from this pectin, but owing to the insolubility of pectic compounds in hydrochloric acid, they are not removed by this treatment from the tissues, which continue to stain deeply with ruthenium red. The more pronounced effect on the pectic structures produced by increasing the concentration of the hydrochloric acid is attributable to its decomposing action with production of derivatives of non-pectic character. Subsequent boiling with caustic alkalis, however, causes disintegration of the tissues and entire absence of staining, since both pectin and pectic acid and the middle lamella pectic constituents are soluble in caustic alkalis, after previous treatment with hydrochloric acid. Boiling ammonium oxalate has much the same effect as hydrochloric acid followed by caustic alkalis, being a very efficient solvent for all the pectic constituents of the tissues.

VII. OBSERVATIONS ON THE PECTIC CONSTITUENTS OF PLANT TISSUES OTHER THAN THE APPLE.

The tissues of a limited number of plants representative of various types of plant structures have been examined, with a view to comparing the distribution and appearance of their pectic components with those found in apple tissue.

It was found that important differences occurred in the majority of the specimens examined, the structures on the whole being dissimilar to those

found in the apple. For instance, in the potato both crescents and discs were located in the cell-walls, but these were very minute and reacted feebly with ruthenium red. On the other hand, the middle lamella was distinctly outlined and the cell-walls did not exhibit the unequal staining so characteristic of those of the apple. These features in the potato are possibly related to the fact that the tissue of the potato is compact and the intercellular spaces are relatively small. In certain swollen root-structures such as the turnip and carrot, in the petiole of rhubarb, and in certain fruits, such as the orange, lemon, plum, gooseberry, and red currant, an abundance of pectic material which stained deeply with ruthenium red was observed in the cell-wall and middle lamella.

It was observed also that the development of the gooseberry fruit, which was followed from the time of fruit setting to ripening, was not attended by any special modifications of the pectic framework, that is to say, no crescents, discs, nor globules were discernible.

The presence of pectic compounds was confirmed in certain specialized tissue structures; for example, in the collenchyma of rhubarb, the epidermal and hypodermal cells and the cells of the parchment layer bounding the ovarian cavity in the black currant, the larger cells of gooseberry, and the small cells of the rind of the orange, lemon, and grape-fruit.

The older phloem elements in various plants often stain intensely with ruthenium red.

A special development of pectic substance is often found to be associated with the comparatively large pits which occur in the thick-walled cells, and notably in the irregular cells of the ground tissue of the rind of orange, lemon, and grape-fruit. When treated with ruthenium red and viewed from certain directions, the pits show up as structures of crescentic outline which recall the crescents observed in the apple fruit.

Mangin (38-45) showed that the formation of intercellular spaces in plant tissues was associated with various modifications in the pectic components of the cell framework, which he describes as 'boutons', 'bâtonnets', 'cadres d'union', &c. In the case of the melon and *Narcissus* it has been found that such modifications as Mangin describes do actually occur. With the melon various stages in the process of cell separation were observed. In the early stages the cells are held together by short cylindrical connexions which consist of intensely staining pectic substance ('cadres d'union'), and as development proceeds, these connecting strands become constricted and are finally ruptured as the tissue attains its maximum expansion. In consequence of this rupture large globules of pectic substance remain on the opposed walls of the separated cells ('boutons', 'bâtonnets').

VIII. PECTIC CHANGES ASSOCIATED WITH ABNORMAL CONDITIONS IN THE APPLE.

The work on normal apples has been extended to an examination of pectic changes in apples in abnormal states, namely, apples affected by functional and fungal diseases and apples subjected to artificially induced conditions, such as exposure to gases, chloroform, &c.

(a) *Functional Diseases.*

Considerable confusion exists at the present time as to the classification of the functional diseases of the apple, especially those which make their appearance in apples held under low temperature or gas storage conditions. In all countries where the conservation of apples is carried out on a large scale under these conditions it is not an uncommon experience to find that a certain proportion decay prematurely. The tissues of the apples become brown, and in some cases tissue-softening ensues. The symptoms of decay may be confined to the general cortex, or they may extend from the cortex to the skin, in which case disease is evident at the exterior of the apple. The actual macroscopical symptoms are very variable: in some cases the brown discoloration is fairly generally distributed, in others it is confined to the central region of the apple, and in still other cases the tissues bordering the vascular bundles are prominently discoloured. The brown areas are sharply defined in some cases, whilst in others they merge gradually into the uncoloured tissues. Cavities may or may not be present in the brown flesh, and the formation of cavities may precede the development of brown areas. Different varieties of apple are not affected alike when held under similar storage conditions. Again, the same variety of apple may exhibit different symptoms in different years although the storage conditions have remained practically unaltered.

Reference to the literature relating to the various functional troubles shows that the classification attempted has been based almost entirely on macroscopical characters, microscopical characters being rarely taken into account. It is thus practically impossible to compare accurately one form of functional disease with another. Again, the macroscopical descriptions are often incomplete; thus in describing a particular functional disease it is often not stated whether the tissues are soft or firm. Since a condition of softening would be brought about by the solution of the middle lamella consequent upon pectic disturbance, the presence or absence of softening would afford an important clue as to whether the chemical reactions taking place in the different categories of functional disease were similar or otherwise.

For convenience, the classification adopted by Kidd and West (35) has

been followed in describing the pectic changes observed in the various forms of functional disease studied by the writers. •

The following observations should be regarded strictly as of a preliminary nature. The whole question of the symptoms characterizing the functional diseases of the apple needs a much fuller investigation.

1. *Internal break-down* (Pl. XIV, Figs. 30-2). This name is used by Kidd and West to designate certain functional troubles which occur when apples are stored in ventilated chambers under low temperature conditions. Under this name are classified forms of disease where tissue-softening occurs, and forms where the tissues remain firm.

The soft type of break-down is often prevalent among apples stored at 1°-3° C. In the Bramley's Seedling variety it is usually evident at the exterior of the apple; the skin becomes discoloured and the sub-cuticular tissue yields readily to pressure. The first signs of the disorder are usually to be detected in the peripheral region of the cortex, whence the disease spreads until the whole apple is rotten. The condition is illustrated in Text-fig. 7. A microscopical examination of an apple affected with this soft break-down at the mid-period of storage yields the following results. In the brown region of the cortex the pectic substances of the cell-walls exhibit change and have completely disappeared from the oldest brown cells. The middle lamella is dissolved and stringy masses of pectic material occur between the cells (Pl. XIV, Fig. 32). The tissue at the margin of the brown area exhibits intermediate pectic changes. The crescents and discs usually show advanced stages of decomposition. The cell-wall pectic substances are unevenly distributed and globules of various kinds are prevalent. As the sound tissue is approached, the pectic disturbances become less and less evident, and in the healthy tissue no abnormal changes are found.

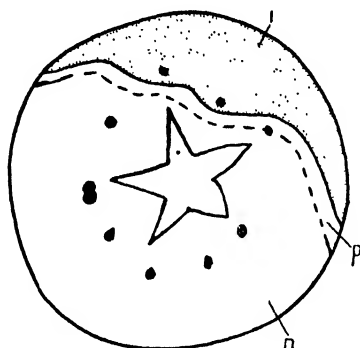
This series of pectic changes is entirely analogous to those changes prevailing during normal senescence, differing mainly in the rapidity with which the changes are effected, and also in the completeness of the pectic decomposition attained, which in normal apples is observed only in the most extreme stages of senescence, such as are rarely found owing to the prevalence of fungal invasion.

The later the onset of internal break-down, the less is found to be the contrast between the condition of the pectic substances in the sound and diseased portions of the apple, because the normal changes are trending in the same direction as those which are taking place prematurely in internal break-down.

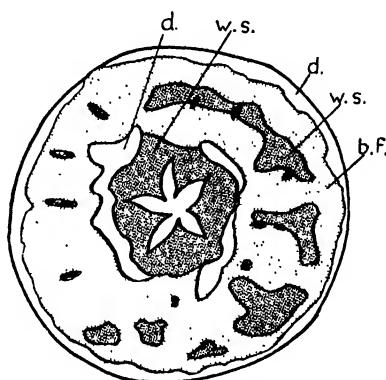
It is not possible to make an exact differentiation between apples affected with internal break-down and apples in a state of natural decay. Generally speaking, in the former case the trouble spreads inwards from sub-epidermal and local areas of the cortex and the tissues are brown, soft,

and watery; in the latter case the cortex is more or less uniformly 'mealy', and the tissues are not always discoloured when the cortex is in a state of 'mealiness'.

It was observed that the pectic framework is not necessarily uniformly affected by the processes which bring about the hydrolysis of the pectic substances of which it is composed. Thus in some cases the crescents and



TEXT-FIG. 7.



TEXT-FIG. 8.

TEXT-FIG. 7. Transverse section of an apple affected with internal break-down (median plane), showing diseased areas. *n*, normal tissue; *p*, zone where partial pectic changes occur; *i*, brown soft tissue where advanced and final changes are found.

TEXT-FIG. 8. Transverse section of a Jonathan apple (U.S.A.) affected with internal browning (December 9, 1925), median plane. *w.s.*, dark water-soaked tissue; *b.f.*, brown firm tissue; *a.*, slightly discoloured tissues. Disease not evident at exterior of apple.

discs have undergone solution before the middle lamella is decomposed; in others the reverse has happened, when unaltered crescents and discs may be observed associated with the walls of completely separated cells.

On some occasions when apparently healthy apples were examined it was found that certain areas in the peripheral cortex exhibited a more advanced state of pectic change than was evident in the surrounding tissues. These areas may be interpreted as centres of incipient internal break-down, and it may be possible therefore to predict the imminence of internal break-down in stored apples by examining a sample microscopically.

The firm type of internal break-down was found in Bramley's Seedling apples kept in cold storage at -1°C . at the Low Temperature Station, Cambridge. These apples, for which the writers are indebted to Dr. West, were examined in April, 1924. The apples were green to greenish yellow in colour and showed externally no signs of disease. On cutting them open the cortex was found to be pale brown in colour. The whole of the cortex was affected, with the exception of a narrow zone of cells beneath the skin. A microscopical examination showed that the relatively slight browning was due to the fact that uncoloured living and brown dead cells occurred

intimately intermingled throughout the tissues. A comparison with normal apples stored at 1° C. showed that comparatively little abnormal pectic change had taken place. The middle lamella was intact, which accounted for the firmness of the tissues. Bottle-shaped cells, which are usually a striking feature in tissues affected with the soft type of internal break-down, were almost absent. The condition of the dead cells somewhat resembled that found in apples which have been exposed to chloroform vapour for a short period, in which a more prominent development of bands is the only sign of any abnormal pectic change. The symptoms described above, namely, the firmness of the tissues, the peculiar distribution of the dead and living cells, and the unaltered cell-walls, correspond very closely with those described by Winkler (68) as present in apples affected with 'internal browning', a functional disease sometimes prevalent among apples when stored under low temperature conditions ($-1^{\circ}\text{C. to } +5^{\circ}\text{C.}$) in the United States.

In the autumn of 1925 the authors obtained some specimens of Jonathan apples which were traced to a shipment from Washington. These apples were firm, green, and entirely free from external symptoms of disease, but internally they were diseased to such an extent that extremely little sound tissue remained. Text-fig. 8 shows the general features which were observed when one of these apples was cut in half. The general mass of the tissues (*b.f.*) was of a medium brown colour. The brown tissue extended to within a few millimetres of the skin, leaving a narrow, slightly discoloured zone (*d.*). Water-soaked areas (*w.s.*) were present in the tissues, the area in the centre was light brown, the remainder very dark brown in colour. These general symptoms agree with those described by Winkler (68) as characteristic of the late stages of 'internal browning'. The condition of the tissues in this advanced state of disease was still unassociated with any extensive disturbance of the normal sequence of pectic changes. Typical cells from the diseased tissues of the apple illustrated in Text-fig. 8 are represented in Pl. XIV, Figs. 33 and 34.

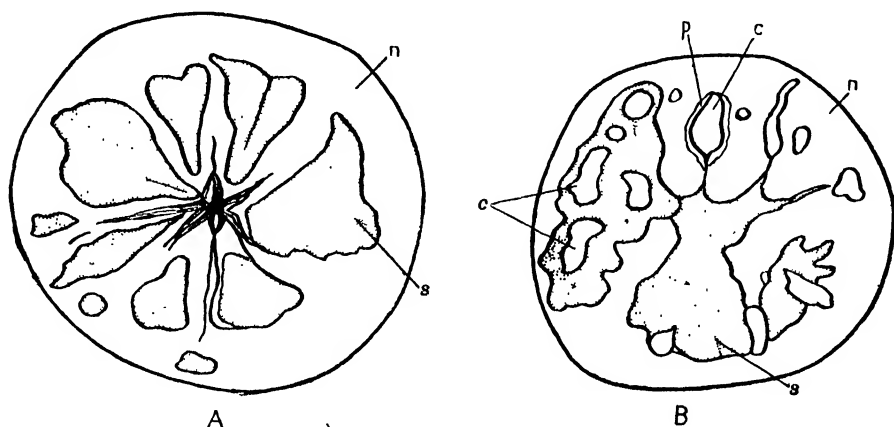
2. *Scald* (see Brooks and Cooley (7)). Under this category, Kidd and West include (α) browning of the superficial cells of the apple unaccompanied by softening or browning of the flesh, and (β) the various 'spotting' diseases of apples which are not due to fungi.

The authors' observations have been confined to cases of typical scald (α). The troubles included under (β) also include spotting due to the presence of sub-cuticular, bitter-pit areas (28-9) which should be classified under bitter pit.

In the cases of scald examined, the skin of the apple was locally discoloured and sometimes slightly indented. This appearance is due to the death and browning of epidermal and of one or more layers of sub-epidermal cells. Very little difference was detected between the pectic constituents of

the dead and those of similarly situated normal cells. The parenchymatous cells adjacent to the affected tissue, however, exhibited partial pectic changes—altered crescents, globule formation, &c. The middle lamella was not dissolved.

Apples affected with scald frequently develop tissue-softening when held under cold storage conditions after scald has appeared. The softening commences in the tissue underlying the layer of dead cells of scald origin and spreads internally. The microscopical effects produced are identical with those associated with internal break-down.



TEXT-FIG. 9. Transverse sections (median plane) of a Newtown apple affected with brown heart under gas storage conditions (May 9, 1924). (A) Section of an apple taken 2.5 cm. from the stalk end. (B) Section of an apple taken 1.5 cm. from the flower end of the same apple. *c*, cavities; *s*, soft brown tissues; *n*, normal tissue; *p*, zone of partial pectic disturbance.

3. *Brown Heart.* Under this heading, Kidd and West (36) describe a functional disease which is encountered among apples held in gas storage. The apples are affected with tissue browning which is usually not evident until the apples are cut open (Text-fig. 9, A and B). Large cavities¹ (Text-fig. 9, B) in the flesh are of common occurrence, attributed by Kidd and West to the 'drying out' of the decaying tissues. The following microscopical details were observed in a small sample of apples for which the authors are indebted to Dr. Kidd. Both softened tissue regions and cavities were present in the specimens received. The softened tissue exhibited the pectic disturbances characteristic of apples affected with internal break-down—hydration and disappearance of the crescents, extreme development of globules of pectic substance, and dissociation of the cells, with the usual formation of bottle-shaped cells. Each cavity was bordered by a narrow layer of crushed cells, the walls of which were impregnated with pectic sub-

¹ The formation of cavities or hollows in the flesh of apples is of course not confined to those affected with 'brown heart'. Cavities may be induced by submitting apples to various experimental conditions.

stance. The living cells surrounding the crushed layer exhibited partial pectic disturbance as seen in the cells bounding a bitter-pit area.

4. *Bitter Pit.* Pectic changes of various kinds occur in tissues affected with bitter pit. The cells in a typical bitter-pit area are dead and shrivelled. Occasionally a wound cambium is present in the neighbourhood of the dead cells, the new cells formed often projecting in a filamentous manner into the air-spaces. The walls of the dead cells stain intensely with ruthenium red, but the discs, crescents, and globules have disappeared. The cells do not separate, the rents in the tissue appear to be produced mechanically through cell contraction. Partial pectic changes are found in the narrow zone of cells surrounding the diseased area.

5. *Water Core* (see J. B. S. Norton, 'Phytopathology I', pp. 126-8, 1911; P. J. O'Gara, 'Phytopathology III', pp. 121-8, 1913; F. D. Heald (27), and others). The tissues in the water-soaked region of apples affected with water core were examined with a view to ascertaining to what extent, if at all, the infiltration of the intercellular spaces by water had brought about changes in the pectic constituents of the cells. No changes which could be associated with the waterlogged condition were detected.

It follows from the foregoing account that considerable differences are observed in the pectic disturbances obtaining in the different functional diseases. These differences may be attributed to variations in the factors promoting pectic decomposition, peculiar to the disease in question, since it is probable that the above-described diseases are due to abnormalities in the metabolic processes, thereby producing variability in the mechanisms capable of causing decomposition of the pectic constituents of the tissues. These abnormalities may be associated with the observed variations in the decomposition of the pectic substances, especially in view of the inherent differences which exist in their composition.

(b) *Artificially induced Conditions.*

Certain pectic changes are induced by tissue lesions, Pl. XIV, Figs. 39-41, provided that the material subjected to lesion is obtained from mature apples in which the pectic constituents are already existing in a condition of incipient decomposition.

The pectic disturbance is confined to a narrow zone of tissue surrounding the cut surface. The discs and crescents in this area show marked signs of alteration, and large globules are of common occurrence. The middle lamella remains unchanged.

Similar and more pronounced changes are produced by the action of gases. Whole apples (Bramley's Seedling) in which incisions had been made were submitted to the atmosphere of various gases in sealed jars, except in the case of ozone, when a special ozonizer was used. Precautions

were taken to ensure that the observed changes were actually produced *in addition* to those resulting from lesion.

In the case of oxygen and ozone the changes do not differ markedly from those due to tissue lesions, and are limited to a narrow zone of cells surrounding the cut surface. The discs and crescents are altered, prominent bands occur, and globules are prevalent. The middle lamella appears to be unaffected.

With nitrogen and carbon dioxide the pectic changes are similar to those obtaining in internal break-down (Pl. XIV, Figs. 35-8). The tissue becomes brown and softens, the cells tend to separate, and stringy masses of pectic material are deposited between the cells—globules are prevalent, the discs and crescents disappear, and finally there is considerable diminution of the pectic substances. Somewhat different symptoms were obtained when varieties other than Bramley's Seedling were used. When uncut apples of certain varieties were kept in an atmosphere of CO₂ for some time the skins became discoloured.

When mature apples are exposed to the vapour of chloroform or ether for about two hours, the tissues become brown owing to death of the cells, but there are no marked signs of pectic disturbances. The middle lamella is not affected.

The effect on the pectic substances of these various gases and of lesions is thus widely different. Oxygen and ozone appear to affect only the cells in the cut area in immediate contact with them, and the slight effect produced may be at any rate primarily due to lesion. Chloroform and ether appear to exert an inhibitory action on the mechanisms producing pectic decomposition, and death of the cells results from the treatment. Tissue lesion appears to have the sole effect of accelerating the pectic changes in the immediate area affected. This local effect may be attributed to the liberation of enzymes or cell contents capable of causing decomposition of the pectic substances. The effect of nitrogen and carbon dioxide in accelerating pectic changes is curious, and may possibly be due to the piling up of unoxidized products which directly or indirectly accelerate pectic decomposition.

(c) *Fungal Diseases* (29).

A preliminary study of the effect of fungal invasion upon the pectic constituents has been made. The fungi used included species, such as *Cytosporina ludibunda*, previously found by the authors to be capable of causing decomposition of pectin, together with *Pleospora pomorum* and a species of *Fusarium* which do not appreciably bring about such decomposition.

It was observed, in certain cases of fungal attack (Pl. XIV, Figs. 42-6), that the cells of the host tissue occupying a narrow zone immediately in

advance of the invading organism were dead as evidenced by their refractive index and plasmolysed contents. In tissues unaffected by *Fusarium* and *Pleospora pomorum*, the middle lamella appeared to be intact, and no marked disturbances of the other pectic constituents were manifest either in the zone of dead cells or in the cells in the region occupied by the invading hyphae. Tissues attacked by *Cytosporina ludibunda* showed pectic changes in the zone of dead cells in advance of the fungal hyphae (Pl. XIV, Fig. 42), as evidenced by the presence of crescents in various stages of alteration and by globules of pectic substance. More advanced changes were observed in the tissues actually invaded by the fungus (Pl. XIV, Figs. 43-6), and in some extreme cases an almost complete disappearance of the pectic constituents was observed.

In all the cases examined, partial or complete cell separation is invariably observed in the invaded tissues. This may be entirely or in part a mechanical effect caused by hyphal penetration between the cells. In the case of *Cytosporina*, however, the evidence suggests that cell separation is partly due to the solution of the middle lamella. The disintegrating action of certain bacteria on plant tissues is well known, and this action has been attributed by Winogradsky (64), Marshall Ward (61), de Bary (3), Brown (8), and others to the decomposition of the pectic constituents of the middle lamella by enzymes secreted by the bacteria themselves.

Brown (8) has shown that certain fungi, notably *Botrytis*, are able to excrete a toxic substance which kills the cells of the host. Since in all the cases cited above the host cells are killed in advance of the invading hyphae it is highly probable that the death of these cells is due to a fungal toxin. In those cases of fungal invasion which are not characterized by any marked change in the pectic constituents an effect analogous to that caused by anaesthetics is evident, and it may be assumed that cell-death and the absence of pronounced pectic changes are simultaneous effects produced by the same toxic agent. The case of *Cytosporina ludibunda* is essentially different in that this fungus is capable of causing the decomposition of pectic substances. Not only are pectic disturbances and loss of pectic substance evident in the invaded tissues, but the disturbances extend to the tissues in advance of the invading hyphae. Hence it is not unreasonable to suppose that these disturbances are due to the partial or complete utilization of the pectic substances by the fungus itself as a source of food.

IX. GENERAL DISCUSSION.

It has been shown that the pectic substances are in a state of flux from the end of the flowering period onwards to the period of natural decay. From the setting of the flower to the maturity of the apple the tissues, including the intercellular system of the apple, are developing. The pectic

substances which are uniformly distributed in the middle lamella and cell-walls when the tissue is compact undergo rearrangement, with the result that the middle lamella occupies a smaller area relative to the size of the cell-walls, and a specialized pectic framework is elaborated which comprises pectic structures of definite shape—crescents, discs, globules, &c. This framework differs in structural detail from similar structures observed in many other plants (see Section VII).

During senescence the pectic components of the tissues undergo further changes. They appear to be in a more labile condition, and masses of semi-soluble pectic matter occur in the cell-walls. Globules are developed, comparable to those found by Mangin in other plant tissues, but not associated by him with a particular phase in development. Consequent upon these changes occurs a gradual lessening of the cohesion of the cells and a modification of the intercellular spaces. The changes proceed very slowly, and culminate in complete decomposition of the pectic compounds, resulting in their total disappearance as revealed by staining. At this stage the cells can be separated from one another by slight mechanical pressure owing to solution of the middle lamella. Plasmolysis of the cells is observed, and many of the cells assume a curious bottle-shaped form.

The study of the normal sequence of pectic changes is often obscured by the incidence of various functional diseases. For instance, internal break-down was prevalent to a greater or less extent in all the years during which this investigation was proceeding. In some seasons when internal break-down was less prevalent a certain percentage of the apples reached the condition of natural decay, whilst in other seasons internal break-down ensued at an early stage, so that the great majority of the apples were prematurely and abnormally broken down. Since the apples were obtained from the same orchard each year, and since approximately the same interval of time elapsed between gathering and placing in cold storage, these differences must be attributed to factors affecting the constitution of the apples before gathering. In this connexion it is interesting to note that Winkler (68) found that the development of functional disease in the Yellow Newton apple was influenced by climate, soil, and methods of orchard practice.

It has been found possible, by microchemical treatment, to imitate the changes which occur during senescence, and since such changes have been effected by the use of hydrolytic reagents (see Section VI), they are interpreted as the result of progressive decomposition of the pectic substances by a process analogous to hydrolysis.

It has also been shown that microscopical changes can be correlated with those observed by quantitative chemical methods which have been independently interpreted as due to the hydrolytic decomposition of the pectic substances (see Section V).

The exact nature of the pectic substances constituting the pectic framework during its development, and the series of changes leading to its decomposition, have already been discussed in some detail (see Section V, and also (12)). At least three forms of pectic substance have been detected—pectin and pectose in the cell-walls, and the pectic constituents of the middle lamella. It is probable that many forms of intermediate composition and varying solubilities occur between these three, and the evidence obtained from microscopical investigation also suggests that the pectic constituents of the cell-wall cannot be regarded as simply insoluble pectose which passes gradually into soluble pectin, but rather that transition compounds arise which may be generally referred to as pectinic acids (see (12)).

The following microscopical observations support this hypothesis: (1) When the tissues are treated with various reagents, the pectic substances are not uniformly acted upon; for instance, the crescents are more readily affected than the middle lamella. (2) The development of the crescents, discs, and globules is not attained simultaneously. (3) The transformations of the crescents and discs do not always take place concurrently with the solution of the middle lamella. (4) Tissues in a condition of 'internal break-down' may exhibit solution of the middle lamella before changes in the crescents and discs become apparent or vice versa (see p. 220).

The following attempt at the correlation of observed microscopical phenomena with the pectic metamorphoses as observed by chemical methods must be regarded as provisional, since it has not so far been found possible to identify with certainty the various structures observed microscopically with those pectic compounds ascertained by independent chemical methods to be present in the tissues.

The pectic framework as it exists in the undeveloped fruit may be conveniently dealt with under two headings: (1) The middle lamella pectic complex; (2) The cell-wall pectic complex.

1. The middle lamella pectic complex may be regarded as containing basal molecules of pectic acid, or salts of pectic acid, since throughout development until complete disintegration of the tissues is observed, it has been found possible to carry out an estimation of the middle lamella pectic constituents in terms of pectic acid. The initial stages of middle lamella decomposition may be conceived as involving the splitting off and solution of these groupings combined with the pectic acid molecule. These groupings, being no longer associated with pectic acid, have lost the typical pectic character of staining with ruthenium red, and the residual pectic acid will therefore tend to coalesce into masses of stainable material, and may be the cause of the appearance of the discs observed in the region of the middle lamella (Table V, I, B). The crescents may similarly be interpreted as dense aggregations of this liberated pectic acid which gradually accumulate round a nucleus of some other cell constituent, possibly the protoplasmic cell con-

nexions. Pectic acid is insoluble in acid media, but slowly soluble in water, from which it follows that these water-insoluble discs and crescents may be expected to persist until the acidity of the cell sap diminishes as ripening proceeds. The observed facts show that changes in these pectic structures are very gradual and tend to become more marked as the fruit becomes less acid. The formation of bands (Table V, I, C) associated with the crescents may be attributed to the gradual imbibition of water by the pectic acid residues, ultimately producing the swollen masses which assume the form of large globules (Table V, I, G). Meanwhile decomposition of the pectic acid itself has probably set in, since in the final stages of senescence no pectic substances of middle lamella origin are apparent, and chemical estimations show a decrease in middle lamella pectic substances at this period. This chemical change is made apparent by the gradual decrease in the size of the globules, their corroded appearance, and their diminished capacity for retaining stain (Table V, I, H and J), until finally no traces of the original middle lamella structures remain (Table V, I, K). This interpretation is supported by a comparison of the changes in the various pectic structures as observed microscopically (see Table II), with the chemical changes in the middle lamella pectic complex shown by the graph in Text-fig. 2, c.

2. Meanwhile, in the case of the cell-wall complex, pectose is undergoing a similar series of degradations, but these are more difficult to follow, since the pectin produced by decomposition of pectose is readily soluble, and it is therefore difficult to ascertain its presence by microscopical methods. The appearance of irregular-shaped staining patches (on the free walls), which take the place of the uniformly staining walls of the undeveloped fruit (Table V, II, F), may be interpreted as residual masses of partially changed pectose, the pectin produced often being observed as diffuse stainable masses in the air-spaces or floating in the medium in which the section is examined (Table V, II, E). This was especially marked when sections were examined in alcohol in which pectin is insoluble. The appearance of small globules (Pl. XIII, Fig. 18) and later of larger globules (Table V, II, D and G; also Pl. XIII, Figs. 19 and 23), which are not readily soluble in water, may be attributed to the subsequent decomposition of pectin into the less soluble pectic acid, and the final stages of pectose decomposition will therefore present features entirely analogous to those exhibited during the disintegration of the middle lamella pectic complex (Table V, II, H, J, K.).

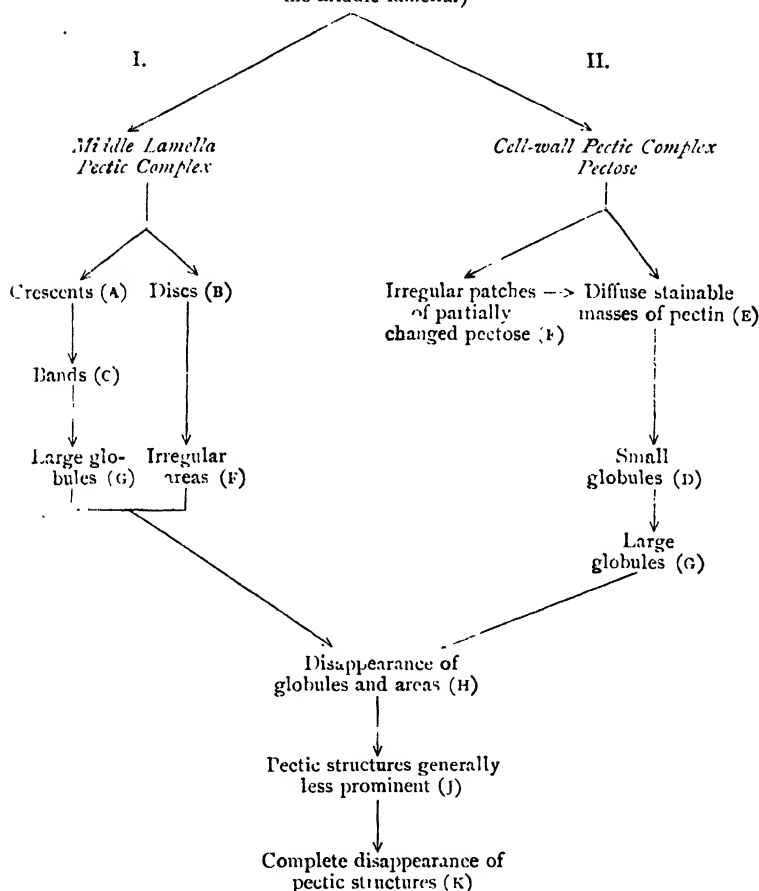
This interpretation of the microscopical phenomena associated with the disintegration of the pectic constituents of the cell-wall is in accordance with the estimated chemical changes, as will be seen by referring to Text-fig. 2.

From a consideration of all the results obtained in the course of these investigations, it is evident that the pectic changes induced in apple tissues

TABLE V.

Schematic Representation of the Origin and Nature of the Various Substances constituting the Pectic Complex.

Initial Pectic Complex.
(i.e. pectic framework comprising the cell-wall pectic substances and those constituting the middle lamella.)



Dissolution of the middle lamella pectic complex with production of difficultly soluble pectic acid (A), (B), gradual swelling of the pectic acid by water imbibition (C), (F), (G), followed by its solution and decomposition (H), (J) into products of non-pectic character (K).

Dissolution of the cell-wall pectic complex with successive production of soluble pectinic acids and pectin (E), leaving areas of modified pectose (F). Decomposition of the pectin (E) into pectic acid (D), (G) with the consequent production of the phenomena observed in (H), (J), (K).

by different agencies are of a somewhat varied character, although exhibiting a similar trend. All the cases of pectic changes may be grouped

together into three classes: (1) Pectic changes involving solution of the middle lamella and general pectic disturbances. These conditions are found in internal break-down (*soft* type), brown heart (regions where soft decay occurs), and apples kept in nitrogen or carbon dioxide. The phenomena observed in these cases are similar to those found in tissues in an advanced state of physiological break-down. (2) Cases where the middle lamella is not dissolved and only partial pectic changes occur. These conditions are associated with lesions, scald, bitter pit (in the cells surrounding the diseased areas), brown heart (in the cells surrounding the cavities), and apples submitted to the action of oxygen or ozone. The changes typical of this class are analogous to those produced in the early stages of senescence. (3) Cases where browning of the cortex is not associated with solution of the middle lamella, and no marked pectic changes occur. This condition is found in the *firm* type of internal break-down (internal browning), in tissues attacked by fungi in advance of the invading mycelium, and in apples exposed to the vapour of ether or chloroform. It is never associated with normal senescence.

The various pectic changes observed in apples, whether associated with the stages of normal senescence or connected with apples in abnormal states, are amenable to the general interpretation, that the pectic decompositions are brought about by enzyme activity under the control of the chemical conditions prevailing in the tissues. It is conceivable that the chemical composition of the unripe fruit exercises an inhibitory effect on the enzymes present, and the gradual changes taking place in the cell contents appear to have the subsidiary effect of stimulating enzyme activity; the pectic changes in consequence become increasingly prevalent, generally leading to the completely disintegrated condition typical of advanced senescence.

In the cases presented by apples in abnormal states, the instances where partial pectic changes occur, as with lesions, may be interpreted as the result of a partial or temporary lessening of protoplasmic control over certain enzymes capable of producing all the pectic changes except middle lamella solution. The phenomena produced in internal break-down (*soft* type), and as a result of exposure to carbon dioxide and nitrogen, may conceivably result from a more extensive and premature action of the factors controlling enzyme activity. In the case of apples submitted to the action of chloroform and ether (where death of the cells does not seem to involve any marked disturbance of the pectic compounds), the anaesthetics appear to have the double effect of killing the cells and of simultaneously arresting the pectic decompositions which normally accompany the death of the cells. The effect produced by anaesthetics supports the view advanced by Winkler to explain internal browning in the Yellow Newton apple. With Bramley's Seedling this form of pathological trouble occurred in apples stored at temperatures below 1° C., whereas the *soft* type of internal break-down occurred at 1° C. and higher temperatures. At

first sight this difference in response might be attributed to temperature. Winkler, however, observed internal browning at temperatures ranging from -1°C. to 5°C. ; hence the phenomenon is not solely related to temperature. Winkler attributes the condition of the tissues in internal browning to the toxic effect of certain organic compounds accumulated at low temperatures. The condition of the cells and the pectic constituents in chloroformed tissue and tissues affected by internal browning are analogous. Hence it is possible that different lethal agents, in one case of an anaesthetic, in the other of a toxin, are able to produce parallel effects. In cases of fungal invasion the same double effect is observed, viz. cell death and little pectic disturbance, and these facts admit of a similar general interpretation, the lethal agent being presumably in this case a toxin of fungal origin.

The disease known as bitter pit may be classed with the cases suggestive of toxic poisoning, the condition of the dead tissue being somewhat analogous to that found in chloroformed tissue, except that, through a process of 'drying out', the cells in the diseased area are ruptured.

Taking into account all the facts brought to light in the course of these investigations, the writers have so far found no ground for supposing that the dissolution of the pectic substances is a primary cause of the death of the tissues. On the contrary, the metabolic changes in the cell contents obtaining in the final stages of senescence appear to have the dual effect of inducing the complete decomposition and solution of the pectic substances and of causing plasmolysis and death of the cells.

X. SUMMARY.

1. In this investigation the results obtained from a microscopical study of the distribution and developmental changes undergone by the various pectic structures in the tissues of the apple fruit are correlated with those accruing from a parallel and purely chemical study of the pectic materials present in apples of the same varieties, derived from the same sources and kept under the same experimental conditions. The results obtained in the two investigations conducted independently are in agreement.

2. The microscopical side of the work has been rendered possible owing to the extreme reliance which can be placed on ruthenium red as a reagent for detecting the presence of pectic substances in apple tissues not previously acted upon by fixatives or preservatives of any kind.

3. From a comparatively simple structure, as observed in the tissue at the time when the fruit is 'set', the pectic complex becomes elaborated during fruit development into a specialized and relatively stable framework, which is revealed as a stainable residue, and amenable to the action of pectic solvents after the cellulose constituents of the cell-wall have been removed by the action of Schweitzer's reagent. During the period of senescence

the pectic framework becomes less stable, and the various structures which constitute the framework may be differentiated from one another by the degree of resistance they offer to the action of pectic solvents. This instability synchronizes with the appearance of soluble pectic derivatives (pectinic acids and pectin) in the expressed juice of the apple, as determined by chemical methods. The quantity of pectin obtained by chemical extractions gradually increases during the period when the less stable constituents of the pectic framework exhibit the maximum degree of change as observed by microscopical methods. At the same time the greater resistance exhibited in time by the more stable constituents of the pectic framework towards solvents is in accordance with expectation, since after the extraction of the soluble pectic compounds less soluble pectic substances may be extracted from the residues by reagents of greater hydrolytic power. From their lack of synchronism, both in development and in the transformations they undergo during senescence, the various structures composing the pectic framework appear to differ among themselves constitutionally, thus supporting the view advanced from the chemical side of the complex nature of the pectic constituents of the cells.

4. All the changes in time exhibited by the less stable pectic structures under normal conditions can be imitated by treating sections of the tissues with hydrolytic reagents. Hence the normal transformations which these structural forms undergo are regarded as brought about by some process of hydrolysis. This view coincides with that already advanced in interpretation of the chemical results.

5. A brief description is given of the pectic framework in the pear fruit and the changes in time which this framework undergoes (Section V. (*f*)).

6. Certain structural forms assumed by the pectic compounds in the apple, e.g. globules, &c., appear to be of common occurrence in plant tissues; other structural forms, crescents, discs, &c., have not been observed in the tissues of the majority of the plant structures examined.

7. The methods adopted in this investigation have been applied to the study of the pectic changes which occur in apples in abnormal states, e.g. physiological and fungal diseases and artificially induced conditions, and certain results of a preliminary nature are given (Section VIII).

The authors are greatly indebted to Professor V. H. Blackman for his kind help and criticism.

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EXPLANATION OF PLATES XII-XIV.

Illustrating Drs. Carré and Horne's paper on an Investigation of the Behaviour of
Pectic Materials in Apples and other Plant Tissues.

The authors are indebted to Miss M. Reeks, Technical Artist in the Imperial College, for the text-figures and plate drawings in this paper. Figs. 2-4 and 6-14 are purely diagrammatic; the remainder are semi-diagrammatic representations of drawings made with the aid of the camera lucida.

PLATE XII.

Fig. 1. Transverse section of young tissue (May 29, 1924) showing small inter-cellular spaces (*s.*) and free walls (*f.*).

Fig. 2. Portion of cell (July 2, 1924) showing the first appearance of discs (*d.*).

Fig. 3. Portion of cell (July 16, 1924) showing bands (*b.*) and discs (*d.*).

Fig. 4. Portion of cell (August, 1924) showing bands (*b.*), discs (*d.*), and crescents (*k.*) in early stages of formation.

Fig. 5. Cell from mature apple (December 4, 1923) showing walls of contact (*w.*), discs (*d.*), bands (*b.*), and crescents (*k.*). Also free walls (*f.*) and small globules (*g.*).

Figs. 6-14. *Series of diagrams illustrating the pectic changes which take place in the region of a wall of contact (w.) during the development and senescence of the apple.*

Fig. 6. Cell (September) showing fully developed discs (*d.*) and crescents (*k.*). Bands (*b.*) absent, free walls (*f.*).

Fig. 7. Senescent changes—crescents (*k.*) showing slight alteration.

Fig. 8. Senescent changes—initial stages of bands (*b.*), crescents (*k.*) are swollen.

Fig. 9. Senescent changes—bands (*b.*) strongly developed, crescents (*k.*) much altered.

Fig. 10. Senescent changes—bands (*b.*), discs (*d.*). Early stages in globule formation (*gg.*). Only outlines of crescents (*k.*) remain. Discs (*d.*) exhibit alteration.

Fig. 11. Senescent changes—further development of large globules (*gg.*). Bands (*b.*) less prominent, discs (*d.*) altered.

Fig. 12. Senescent changes—large globules (*gg.*) prevalent. Bands (*b.*) and discs (*d.*) tend to disappear.

Fig. 13. Senescent changes—remains of large globules (*gg.*). Discs (*d.*) in various stages of disappearance.

Fig. 14. Senescent changes—final stages in the disappearance of pectic substances, remains of bands (*b.*) and discs (*d.*).

PLATE XIII.

Figs. 15–19. *Series of diagrams illustrating the pectic changes which occur during senescence in Bramley's Seedling apples held in storage at laboratory temperature.*

Fig. 15. Cell showing two walls of contact (*w.*) and free wall surface (*f.*), outlines of crescents (*k.*), bands (*b.*), large globules (*gg.*), discs (*d.*).

Fig. 16. Thick transverse section showing the cells bounding an intercellular space (*s.*), free wall surfaces (*f.*), small globules (*g.*), connecting strands of pectic substance (*c.*).

Fig. 17. Transverse section—intercellular space (*s.*), free walls (*f.*). Swollen pectic substance at the re-entrant angles (*r.*). Pectic substance (*c.*) is observed connecting cells i and ii.

Fig. 18. Thick transverse section (December 3, 1925) showing the cells bounding an intercellular space (*s.*) with extensive development of small globules (*g.*) on the free walls (*f.*).

Fig. 19. Another section (November 30, 1923) taken from an apple obtained from the same locality as that used for Fig. 18 showing similar features.

Figs. 20–3. *Series illustrating advanced pectic changes in Bramley's Seedling apples as observed after ten months' storage at 3° C.*

Fig. 20. Thick section (August, 1924), low magnification, showing the general appearance of the tissues, intercellular spaces (*s.*), walls of contact (*w.*), free wall surfaces (*f.*).

Fig. 21. Single cell isolated from tissue illustrated in Fig. 20, high magnification, showing three walls of contact (*w. i.*, *w. ii.*, *w. iii.*), altered discs (*d.*), remains of large globules (*gg.*), free wall (*f.*) showing a few small globules (*g.*).

Fig. 22. Group of cells (August 7, 1924) bounding an intercellular space (*s.*), walls of contact (*w.*); (*w. i.*) showing large globules (*gg.*); (*w. ii.*) and (*w. iii.*) showing remains of globules (*gg.*) and bands (*b.*); free walls (*f.*).

Fig. 23. Thick section showing cells bounding an intercellular space (*s.*), free walls (*f.*) showing globules of various sizes.

Fig. 24. Groups of cells in an advanced state of senescence from the sub-epidermal region of the apple, showing cell separation in the various stages. (a) Cells still united. (b) Cells in various stages of separation.

PLATE XIV.

Fig. 25. Groups of completely separated cells showing plasmolysis of the cell contents and formation of bottle-shaped cells (*b.s.*).

Figs. 26–8. *Series illustrating the action of reagents on pectic substances.*

Fig. 26. Transverse section of young Bramley's Seedling apple (July 25, 1924) soaked in KOH after preliminary soaking in HCl. The intercellular spaces (*s.*) are filled with pectic substance (*p.s.*).

Fig. 27. Transverse section of young Bramley's Seedling apple (August 5, 1924) soaked in 5 per cent. HCl for three days, followed by 50 per cent. HCl one day, and 50 per cent. KOH five days. Masses of pectic substance (*p.*) in the intercellular spaces (*p.s.*) and in the cell-walls (*p.*).

Fig. 28. Transverse section of young Bramley's Seedling apple (June 16, 1924), soaked in HCl, then in KOH, and subsequently boiled in KOH (5 per cent.). The cells separate on the slightest pressure. Note complete absence of pectic substance.

Fig. 29. Transverse section of two cells from a Bismarck apple (April 24, 1924) stored at 1° C., showing natural phenomena comparable to those produced by reagents (cf. Figs. 26–8), namely, stain-

ing patches of irregular shape up to $28 \times 40 \mu$ in size situated in the walls of contact. These patches are presumably due to the alteration of the discs, which in the Bismarck apple are relatively large, reaching 16μ in diameter. The remaining portion of each wall of contact is unstained.

Figs. 30-2. *Series illustrating the pectic changes occurring in Bramley's Seedling apples affected with internal break-down (November 4, 1925) held in storage at laboratory temperature.*

Fig. 30. Single cell showing three walls of contact (*w.*) almost depleted of pectic substance, and feebly staining free wall surface (*f.*) showing various stages in the disappearance of globules (*g.*).

Fig. 31. Single cell in which it is difficult to distinguish the walls of contact owing to a general depletion of the pectic substances. Walls of contact (*w.*) with altered discs (*d.*), free wall surface (*f.*) with globules (*g.*) in various stages of disappearance.

Fig. 32. Very advanced stage. The cells themselves are entirely depleted of pectic substance. Stringy masses of pectic substance (*p.*) are observed between the cells.

Figs. 33-4. *Pectic changes in Jonathan apples affected with internal browning.* Cells from the water-soaked and brown regions. Walls of contact (*w.*) showing little pectic substance; free wall surfaces (*f.*) with globules (*g.*) in various stages. Note that the middle lamella pectic substance is not dissolved.

Figs. 35-8. *Series illustrating the pectic disturbances produced by storage in an atmosphere of carbon dioxide for ten days at laboratory temperature.*

Fig. 35. Transverse section showing pectic substance (*p.*) at the re-entrant angles made by two cells in contact.

Fig. 36. Three separated cells showing connecting strands of pectic substance (*p.c.*) and irregular masses (*p.*) also of pectic nature.

Fig. 37. Single cell showing dome-shaped pectic masses (*p.*) probably arising from rupture of connecting strands.

Fig. 38. Stage in the formation of the stringy masses of pectic substance (*p.*) shown in Fig. 32.

Figs. 39-41. *Series illustrating the pectic changes resulting from a tissue lesion (Bramley's Seedling Apple).*

Fig. 39. Group of cells viewed from above. Intercellular space (*s.*); pectic substance (*p.*) uniting cells i, ii, and iii; globules (*g.*) on free walls (*f.*).

Fig. 40. Transverse section showing bands of pectic substance (*p.*) connecting the cells.

Fig. 41. Transverse section showing intercellular space (*s.*) with globules (*g.*) on the free walls (*f.*).

Figs. 42-6. *Series illustrating the pectic changes caused by fungal attack (Cytosporina ludibunda).*

Fig. 42. *Pectic changes in cells slightly in advance of the invading mycelium.* Intercellular space (*s.*), connecting strands of pectic substance (*p.s.*), discs (*d.*) in various stages of alteration, globules (*g.*) on the free walls (*f.*), walls of contact (*w.*).

Figs. 43-4. *Pectic changes in the tissues occupied by the invading mycelium.*

Fig. 43. Single cell showing walls of contact (*w.*) with remains of discs (*d.*). The pectic substance has almost disappeared from the free walls (*f.*).

Fig. 44. Thick section stained with cotton blue. The mycelium (*my.*) of the fungus surrounds the cells. Appressoria (*a.*), remains of pectic substance (*p.*).

Figs. 45-6. Cells from the infected region of the tissue stained with ruthenium red. Note that the appressoria (*a.*) are deeply stained, indicating the absorption of pectic substances by the fungus. Walls of contact (*w.*), free walls (*f.*), remains of pectic substance (*p.*).

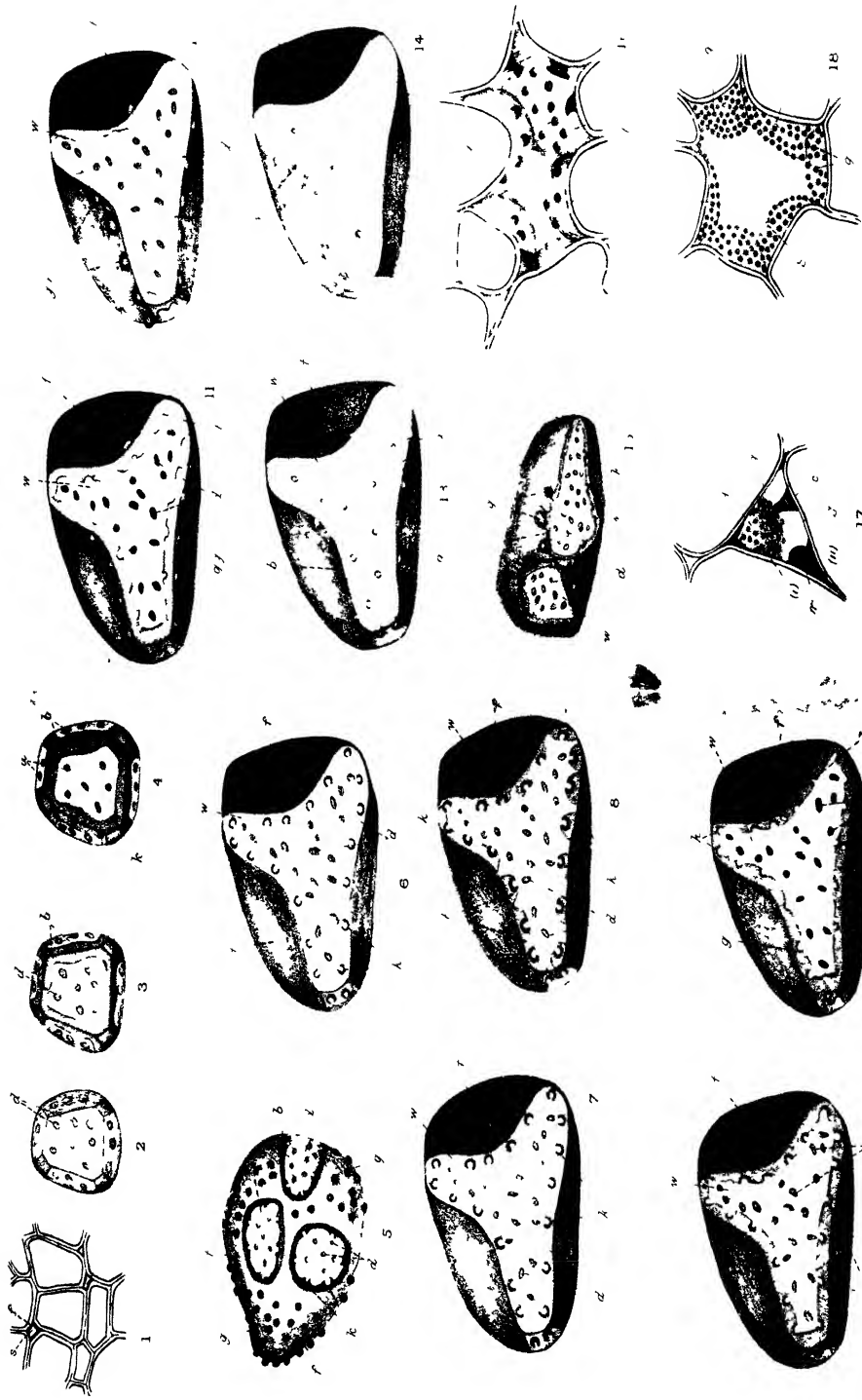
Figs. 47-51. *Series illustrating the distribution of pectic substances in the pear.*

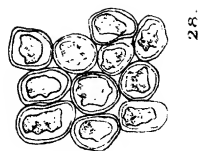
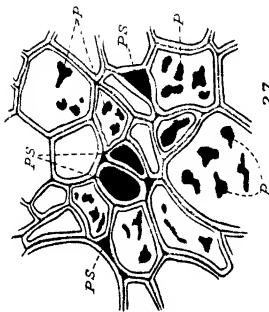
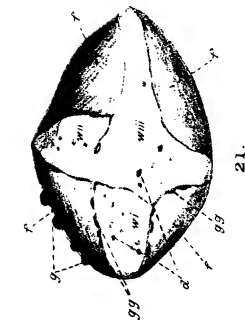
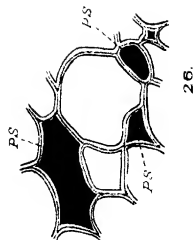
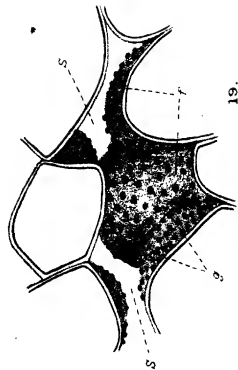
Fig. 47. Single cell from mature pear tissue showing walls of contact (*w.*), free walls (*f.*), discs (*d.*), suggestion of bands (*b.*).

Fig. 48. Cells from tissue in an advanced stage of ripeness, showing walls of contact (*w.*), discs (*d.*), small crescents (*k.*). The free walls (*f.*) present a granular appearance. Intercellular spaces (*s.*).

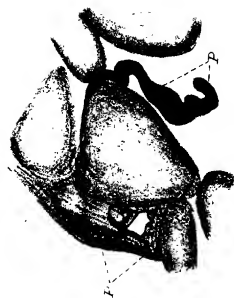
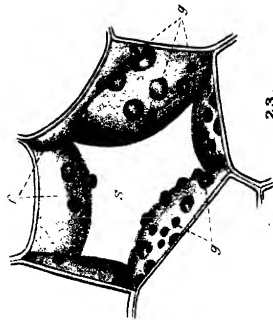
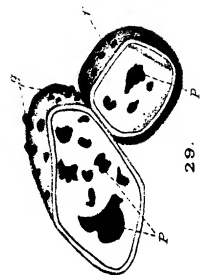
Fig. 49. Senescent changes—group of cells showing initial stages in cell separation. Connecting strands of pectic substance (*p.*), wall of contact (*w.*) showing altered discs (*d.*) and globules (*g.*).

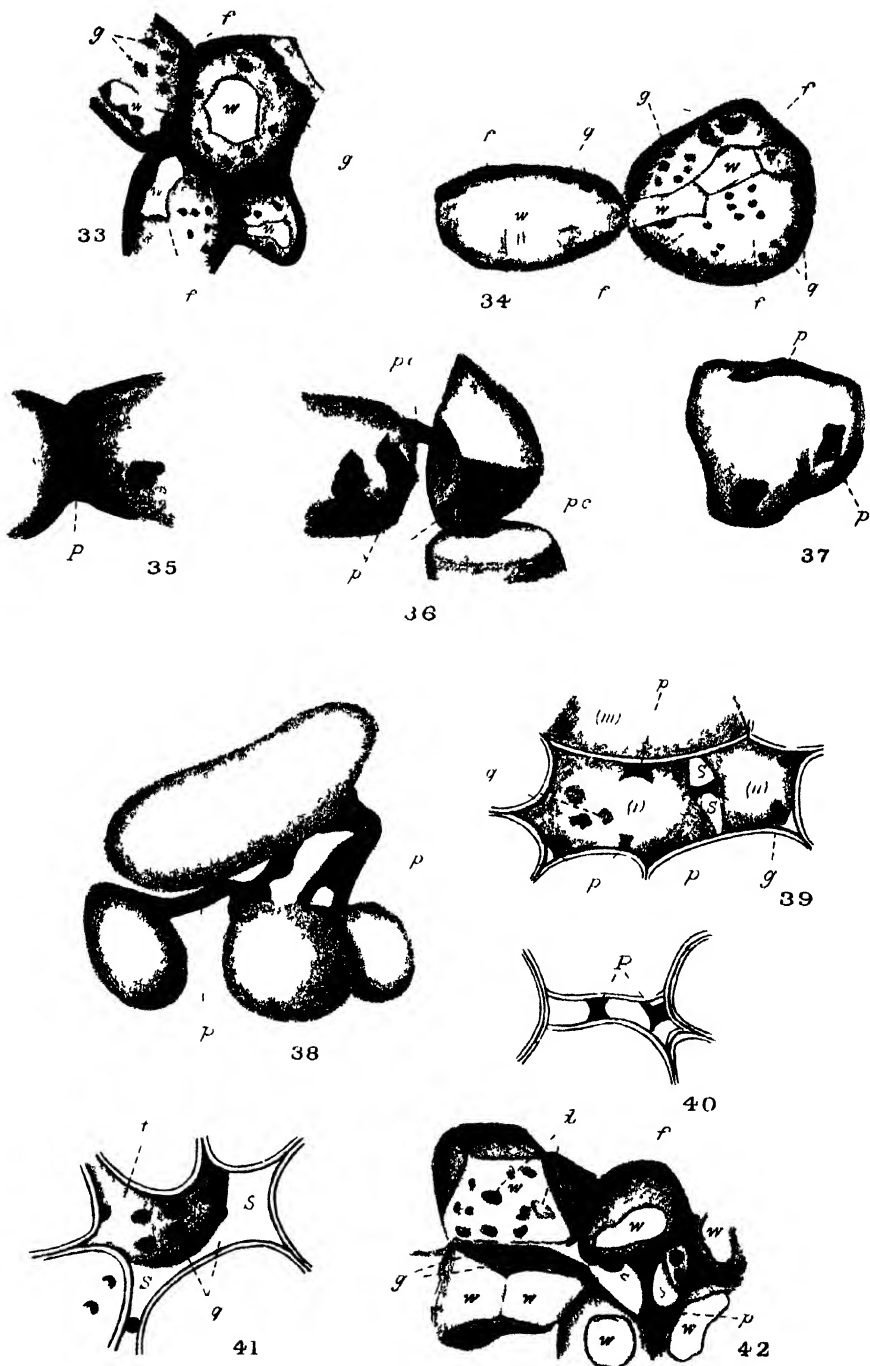
Figs. 50-1. Similar groups of cells showing aggregations of pectic substances (*p.*) on the separated walls and formation of bottle-shaped cells (*b.s.*) and intercellular spaces (*s.*) in Fig. 51 filled with pectic substance (*p.s.*).

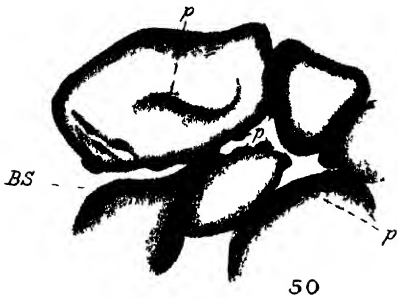
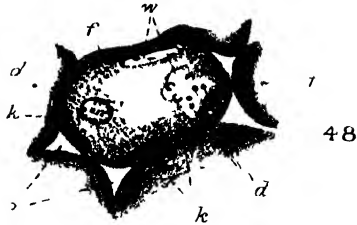
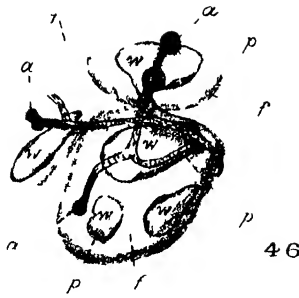
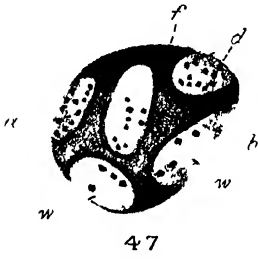
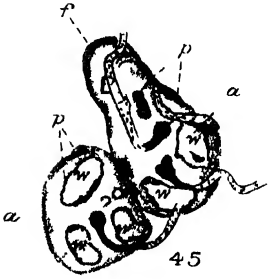
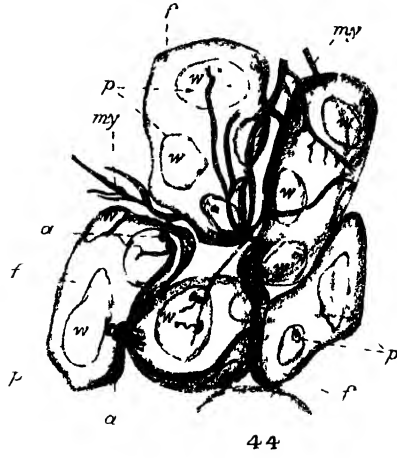
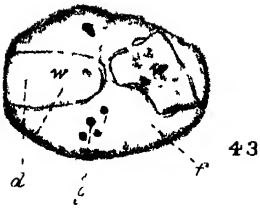




21.







On some Fossil Woods of Mesozoic and Tertiary Age from the Arctic Zone.

BY

JOHN WALTON, M.A.,

Lecturer in Botany at the Victoria University, Manchester.

With Plate XV and four Figures in the Text.

AMONG the fossil plants found by Professor Seward and Mr. Holtum on their expedition in 1921 to the west coast of Greenland are some specimens of Cretaceous and Tertiary fossil woods. Reference is made to these specimens by Professor Seward in his account of the Cretaceous Flora of West Greenland (16, pp. 63, 65), but the work of identification and description was very kindly entrusted to the writer. Thanks to the kindness of Mr. J. M. Wordie, the writer has also had the opportunity of investigating some petrified wood of Jurassic age which Mr. Wordie collected on one of his expeditions to Svalbard (Spitzbergen) and of which a description is given below.

Most of the wood found by Professor Seward and Mr. Holtum is from Cretaceous beds. Professor Seward records the discovery of fossil wood at Atâ and Pâtût (16, pp. 63, 64), but other specimens were found at Kaersuassuk, Kûk, and Skansen. These specimens are for the most part poorly preserved, but enough of their structure is represented to enable one to see that they are all Coniferous and all probably referable to the form-genus *Cupressinoxylon*, Göppert (Gothan emend.) (6, p. 43). Two of these specimens, one from Kûk and the other from Skansen, are moderately well preserved and are described below. In addition, a very fine specimen of wood was found on Hare Island (off the north-west of Disko Island) where Tertiary plants have been found, but none of Cretaceous age.

Cupressinoxylon diskoense, sp. nov.

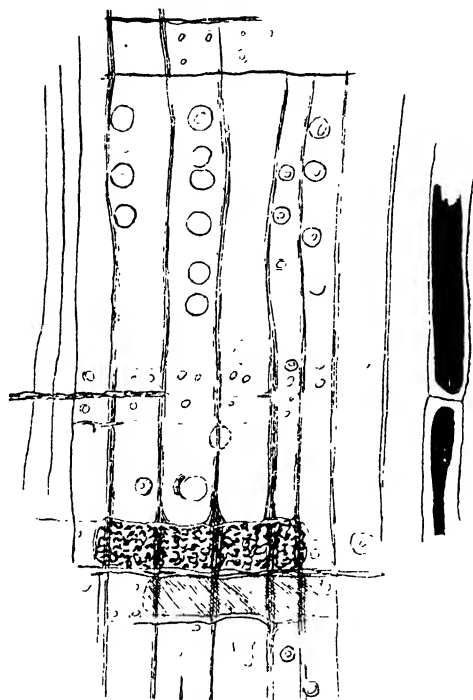
Loose blocks of silicified wood, some better preserved than others, were found in material derived from Cretaceous or Tertiary rocks at Skansen, on the south-east coast of Disko Island. The only fossil plants found at the same locality were in Cretaceous shales, and it is possible that the wood

is also of Cretaceous age. None of the specimens shows any tissues other than secondary wood, and this exhibits the characters of the comprehensive form-genus *Cupressinoxylon*, Kraus (12, p. 374). It is also included in *Cupressinoxylon* in the restricted sense used by Gothan (6, p. 43), on account of the type of medullary ray cell in which the pitting is confined to the radial walls. This is a more satisfactory criterion for distinguishing *Cupressinoxylon* from *Cedroxylon* than the distribution of wood-parenchyma, which is always rather variable (17, pp. 147-8).

Description. In transverse sections (e. g. British Museum, V. 19056), the annual rings are seen to be variable in width and the change from the late wood to the succeeding early wood is quite sharp. The medullary rays are conspicuous on account of the large proportion of resin-containing cells, and appear as thick black lines traversing the wood radially. Resin-containing wood-parenchyma is very abundant and is fairly uniformly distributed throughout the secondary wood. Resin canals are absent. In radial section the medullary rays are seen to be very abundant and to be for the most part uniseriate, although biseriate rays are frequent. They are from one to sixteen cells high. The medullary rays are usually more abundant in *Cupressinoxylon* than in *Cedroxylon*, and Lignier (13, p. 245) has used this as a character of value in discriminating between these two genera. The pitting in the walls of the tracheides consists of bordered pits (diam. 13-17 μ), usually distant and uniseriate, but frequently biseriate and opposite (Pl. XV, Fig. 4). No pits have been observed on the tangential walls of the tracheides. The resiniferous wood-parenchyma has no pits and, like the cells of the rays, is thin walled. The pitting in the field consists of 1-4 simple, slightly oblique pits on the wall of the ray cell, but with a border in the wall of the tracheide (Text-fig. 1). This half-bordered condition recalls the pitting in *Cupressus* and in other living genera of Conifers, where the wall of the ray cell is thin. Each ray cell spans from two to nine tracheides radially.

Cupressinoxyla have already been recorded from Disko Island by Cramer (3, p. 167), *Cupressinoxylon brevernii*, Merkl., and *C. ucranicum*, Göpp. They both differ in having larger pits on the radial walls of the medullary ray cells. The preservation of the type specimens of these two species appears to have been poor, and the pits may have become large through decay. In *C. pulchrum*, Cramer (3, pt. ii, p. 171), from Banks Land the ray pits are still larger. *C. Koettlitzii*, Seward (15, p. 195), resembles our type more closely in this respect, but differs in the absence of resiniferous parenchyma and in the rare occurrence of biseriate rays. Göppert's species *C. nodosum*, from the Cretaceous of Silesia, is somewhat similar to our specimen, but no data are given about the resiniferous parenchyma. *C. McGeei*, Knowlton, from the Potomac Series has similar field-pitting, but the rays are rarely biseriate and the tracheide-pitting is often triseriate.

A specimen from the Upper Jurassic or Lower Cretaceous of King Charles Land has been compared with Knowlton's species by Gothan (7, p. 19). It agrees more closely with our specimen. Schröter (14, p. 17) described a species from the Mackenzie River, Canada, which he termed *Sequoia canadensis*. Gothan's and Schröter's species both exhibit triseriate pitting



TEXT-FIG. 1. *Cupressinoxylon diskoense*, sp. nov. Radial section of the secondary wood. $\times 225$ diams.

and almost entirely uniseriate rays. Beust (2, Table II) tabulates the characters of more than fifty *Cupressinoxyla*, but to none can this specimen be confidently assigned.

Diagnosis. *Cupressinoxylon diskoense*, Walton. Coniferous wood without resin canals. Annual rings distinct; tracheides with uniseriate or biseriate bordered pits distantly placed. When biseriate the pits are opposite. Resiniferous, xylem parenchyma abundant, uniformly distributed. Medullary rays uniseriate to biseriate up to sixteen cells high, often containing resin. Pits in the field 1-4, small, slightly oblique, bordered on the side of the tracheide. Horizontal and tangential walls of the ray cells apparently unpitted.

Locality : Skansen, south-east coast of Disko Island, West Greenland.
Horizon : Probably Cretaceous.

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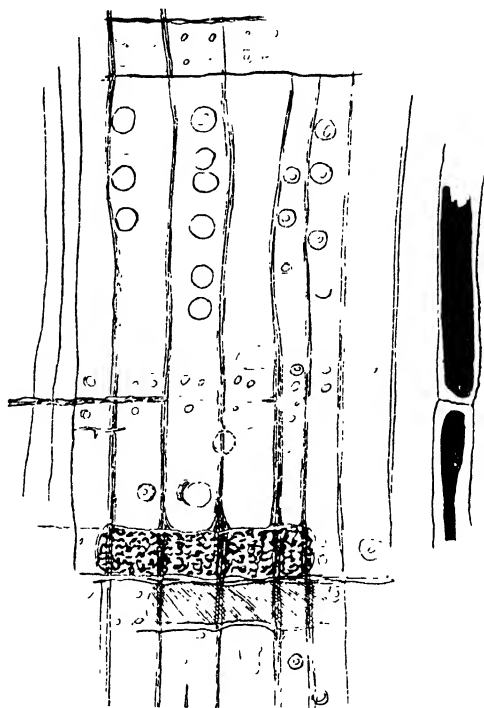
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Locality: Skansen, south-east coast of Disko Island, West Greenland.
Horizon: Probably Cretaceous.

Cupressinoxylon, cf. vectense, Barber.

A small block of hard, calcified, sedimentary material, containing at the centre a piece of petrified wood riddled with *Teredo*-borings, was found at Kûk in Cretaceous beds. There are numerous small fragments of organic material in the specimen (Pl. XV, Fig. 3), including portions of what are possibly fern rachises as well as Foraminifera and Echinoderm spines.¹ The fragment of wood was evidently drifting about before it was embedded along with these fragments of marine organisms near the coast of Cretaceous Greenland.

In transverse section clearly defined rings of growth can be detected. The xylem parenchyma, which is fairly abundant, is scattered throughout the spring and autumn wood. There is no evidence of any resin canals. In longitudinal sections the pitting of the tracheides and medullary ray cells in the better preserved portions resembles that of *C. diskoense*; the greater part of the wood, however, has undergone partial decomposition. As a result the tracheides show steep spiral striations, and the pits in both the tracheides and medullary ray cells appear as large oblique slits with their major axis parallel to the direction of the striations. Pits on the tangential walls of the tracheides are not common. The medullary rays are not numerous as in *C. diskoense*. In the possession of these features, as well as those described above, it resembles *C. vectense*, Barber, very closely.

Locality: Kûk, Nûgssuak Peninsula, West Greenland.

Horizon: Cretaceous.

Cedroxylon greenlandicum, sp. nov.

A piece of silicified coniferous tree-trunk was found by Professor Seward and Mr. Holtum in the form of a loose block on Hare Island, which lies off the north-west coast of Disko Island, West Greenland, lat. 70° 50' N.² The specimen is cylindrical and measures 25 cm. long and 19 cm. in diameter. On one end of the block are a series of concentric ridges formed by the unequal resisting powers to weathering processes of the spring and summer constituents of the seasonal increments of secondary wood. At one point on the surface of the block there is a scar where the vascular supply to a small branch passes through the secondary wood of the stem.

Anatomical features. The pith, approximately circular in transverse section, has a diameter of about 1 mm. (Pl. XV, Figs. 1, 2), and consists entirely of cylindrical cells (about 240 μ long and up to 70 μ in diameter) with thick walls. There are numerous simple pits in the walls of the pith cells and some of the latter contain resin. The cells of the pith which lie next to the xylem are longer and narrower than the others.

¹ Kindly identified by Mr. J. W. Jackson, F.G.S.

² Beust, 1885, p. 16, describes *Dadoxylon Heeri*, Beust, sp., and *Cupressinoxylon Sabiniana*, Heer, sp., from Hare Island.

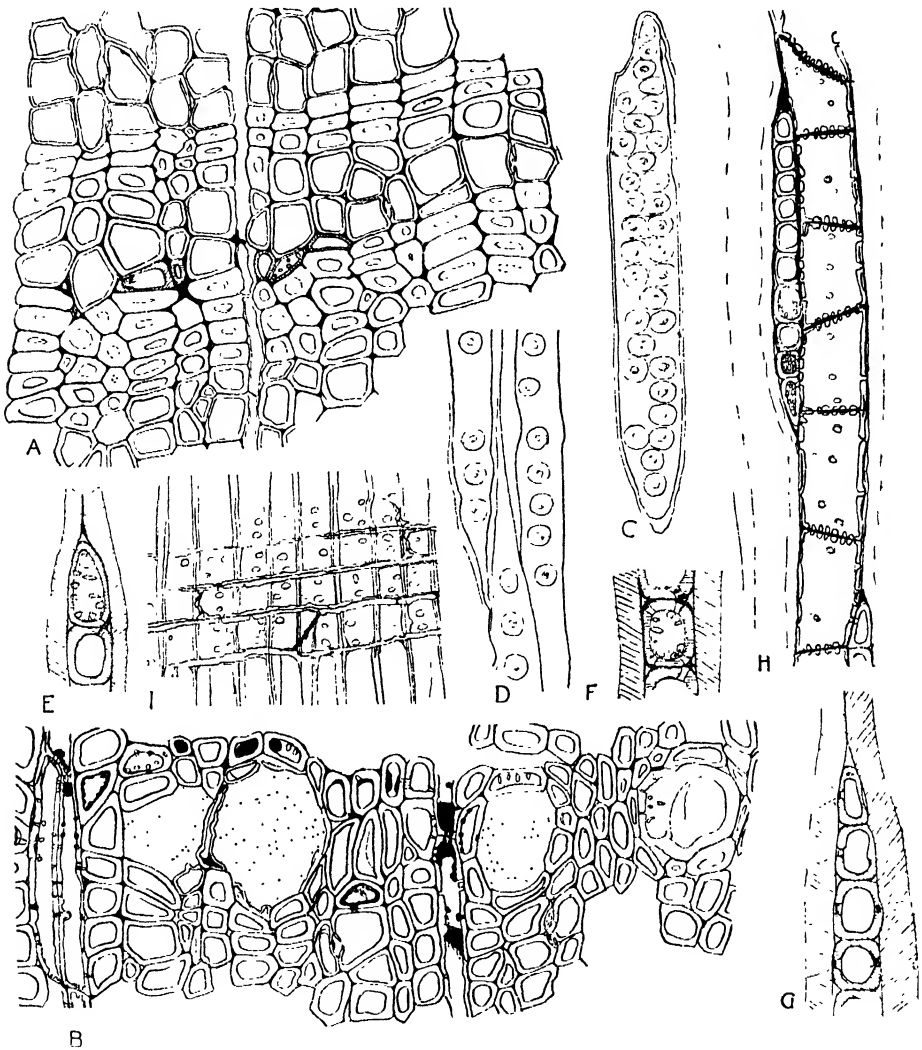
In transverse sections the pith cells are seen to be separated at the corners, where there are small intercellular passages (4, cf. p. 3, Text-fig. 2). In transverse section there are seen to be about fifteen groups of protoxylem tracheides which project slightly into the pith and are invested on that side by the longer and narrower pith cells which have been already described. In longitudinal sections one can see that the xylem is entirely centrifugal. There appears to be no transfusion tissue. The protoxylem elements have a fine spiral thickening; the later differentiated tracheides have reticulate thickening, while the tracheides of the secondary wood have bordered pits.

The secondary wood shows well-defined annual rings (Text-fig. 2, A, and Pl. XV, Figs. 1 and 2) which vary in breadth from very narrow rings to rings about 2 mm. in width. There is a disturbance in the rings due to the presence of a branch (Pl. XV, Fig. 1, bottom left-hand corner). In transverse section (Text-fig. 2, A, B) the tracheides of the spring wood appear approximately square with relatively thin walls, while those of the summer wood are compressed radially and appear rectangular with thick walls and correspondingly small lumina. There is a sharp change from the late wood to the wood succeeding it, and the last elements of the late wood regularly consist of thick-walled parenchyma (Text-fig. 2, A, II), which can be distinguished from the tracheides in transverse sections by the slightly darker colour of the walls and the pitting on the transverse walls whenever these are visible. The last differentiated tracheides in the late wood, though typically pitted on the radial walls, are sometimes pitted on the tangential walls as well. The bordered pits frequently show the torus appressed to the pore on one side of the pit. In radial sections the tracheides in the late wood (Text-fig. 2, B) exhibit a single series of bordered pits (diam. 15μ) with a centric circular pore (diam. 5μ). In the early wood the pitting is uniseriate to biseriate (Text-fig. 2, C); when biseriate the pits (diam. 18μ) are opposite and contiguous. The medullary rays are uniseriate (Text-fig. 2, E, F, G) and vary from one to nine cells in height. The ray cells are thick walled, with small simple pits on all the walls. There are from one to four of these pits in the field (Text-fig. 2, I). Each ray cell spans from three to twelve tracheides radially.

On the side of the stem on which the branch arises there is a considerable development of parenchyma (traumatic?) and in the same region there is a small group of resin canals (Text-fig. 2, B). It was not possible to observe these canals in longitudinal section, so that their vertical extent is not known.

The stem clearly belongs to a tree of Abietinean affinities agreeing most closely with *Cedrus*, among living genera, in the structure of the rays and in possessing traumatic resin canals. Several fossil woods have been described from the Arctic Zone which have been referred to *Cedroxylon*. A closely similar wood has been described by Gothan. This wood, *Cedro-*

xylon transiens, Gothan (7, p. 26, Text-fig. 14, 15, &c.), has been recorded from an Upper Jurassic or Lower Cretaceous horizon in King Charles Land



TEXT-FIG. 2. *Cedroxylon greenlandicum*, sp. nov. A, transverse section of the secondary wood; the walls of the wood-parenchyma are shaded. B, transverse section of a part of the stem with resin canals; the pitting of the medullary rays is also shown. C, radial section of a tracheide of the spring wood. D, radial section of parts of three tracheides of the summer wood. E, F, G, tangential sections of medullary ray cells to show the nature of the pitting. H, tangential section of a medullary ray and a vertical series of wood-parenchyma cells with pits on their tangential, as well as on their horizontal and radial, walls. I, radial section of a medullary ray to show the pitting in the field. Magnification: A $\times 208$; B and I $\times 198$; C, D, and H $\times 154$; E, F, and G $\times 305$. A and B, British Museum, No. V. 19050; C, D, and I, No. V. 19051; E, F, G, and H, No. V. 19052.

and from an Upper Jurassic horizon on Mt. Wiman on the south side of Sassen Bay in Svalbard (8, p. 38); in most of the details of structure there

is a close resemblance to our specimen. The similarity in the structure of the wood-parenchyma is most striking (cf. Text-fig. 2, II, with Gothan (7), p. 29, Fig. 15), and so also the structure of the rays (cf. Gothan (8), Taf. 6, Figs. 11, 12, 13). An important difference exists in the tracheide-pitting, which is distinctly Araucarian in *C. transiens*. The Hare Island specimen agrees most closely with *Protopiceoxylon Johnseni*, Schröter, sp., from King Charles Land (14, p. 15) in the majority of its minute details, but in *P. Johnseni*, Schröter, sp., the resin canals are apparently a normal feature of the secondary wood. Like the latter, the Hare Island specimen has thick-walled pitted cells in the pith. Edwards (4, p. 1) has recently re-described some of Schröter's type sections and has increased our knowledge of its structure. He finds that there is less xylem parenchyma present than Schröter's description leads one to believe. Göppert (5, p. 199) has described several *Cedroxyla* of Cretaceous age from central Europe. Of these, *C. pachyderma*, Göppert, agrees in some respects with our specimen, and *C. Matsumurae* and *C. yendoi*, Stopes and Fujii (18, p. 42), from the Cretaceous of Japan, are related forms.

This species compares very closely with *Protopiceoxylon Johnseni*, Schröter, sp., but is distinguished from it by the absence of normal resin canals in the secondary wood. The absence of 'normal' resin canals is a difference the value of which as a specific distinction is somewhat doubtful in view of the uncertainty of deciding whether a canal has been produced by traumatic stimulus or not. It is therefore also difficult to decide whether a wood belongs to *Protopiceoxylon* or to *Cedroxylon*. The two genera merge into one another (cf. 4, p. 6).

It is clear that secondary wood of this type makes its appearance at several points in the fossil record from Jurassic times to the present day.

Diagnosis: *Cedroxylon greenlandicum*, sp. nov.

Pith consisting of cylindrical thick-walled cells with numerous simple pits. Xylem entirely centrifugal. Secondary wood with well-defined annual rings. Traumatic resin canals present. Tracheides of the late wood, occasionally with tangential pitting, normally with a single series of distant bordered pits (diam. $1.5\ \mu$). The larger tracheides have one to two series of bordered pits; when two series are present the pits are opposite and contiguous. A certain proportion of the late wood consists of thick-walled parenchyma. Medullary rays uniseriate, one to nine cells in height, with thick walls and with numerous simple pits on all the walls. There are one to four pits in each field.

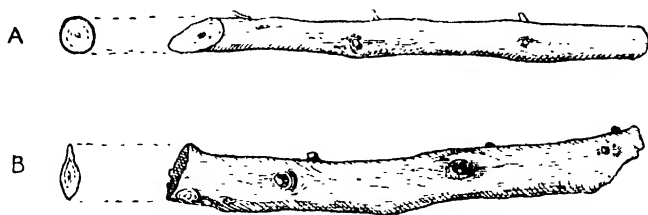
Locality: Hare Island, West Greenland. Horizon ? Tertiary.

Cedroxylon greenlandicum, Walton.

Some lignite was found in Tertiary beds on Hare Island, and from it microtome sections were cut in celloidin. Mr. Holtum, who made the

sections, kindly gave them to me for investigation. In some of these sections cut from a decorticated twig it was possible to distinguish the following features.

The pith consists of thick-walled, abundantly pitted parenchyma, identical in structure to that already described in the petrified specimen. The tracheides are pitted with large uniseriate distant pits. The medullary ray cells are thick walled and are pitted with small simple pits on all the walls. One of the small twigs is shown in Text-fig. 3, B. There are several scars and stumps of small branches arranged distichously. A section cut from one of these small lateral shoots has a small lacunae, probably resin



TEXT-FIG. 3. A, small, decorticated twig of *Cedrus atlantica*, showing the distichously placed vascular supplies to the spur-shoots. B, small twig of *Cedroxylon greenlandicum*, sp. nov., from the lignite on Hare Island, showing the distichous arrangement of the lateral vascular supplies. Natural size.

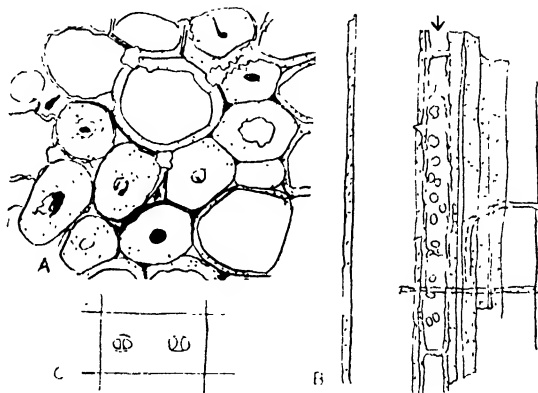
canals in the secondary wood, which is infested with septate fungal hyphae, and the pith consists of thick-walled pitted elements.¹ A twig of *Cedrus atlantica* (Text-fig. 3, A) was stripped and dried, and comparison with the fossil twig shows that they are alike in some important respects. In both there is the distichous arrangement of laterals (short shoots in the case of *Cedrus atlantica*), and in sections cut from corresponding parts there was a general resemblance between the two. Several coniferous genera, however, show the same general relations in their branchings, and it would therefore be impossible to base any deductions as to the relationship between the fossil and any one of these living genera. But these facts, taken along with the structure of the secondary wood, afford a striking basis for comparison between the fossil and *Cedrus*.

Protopiceoxylon Wordii, sp. nov.

Among the specimens collected by Mr. Wordie are some blocks of very well-preserved wood with, in two instances, the pith or part of the pith represented. The pith consists of fairly thick-walled parenchyma in which there are scattered groups of cells with the appearance of stone cells (Text-fig. 4, A) possessing a very thick wall and a correspondingly small lumen. In longitudinal section they are seen to be about three or four times as long as broad and to have simple pits in their walls. From the structure of the

¹ British Museum, V. 19066-7.

centre of the specimen, including the pith, it is clear that we are dealing with stem-wood and not with that of a root. The seasonal rings are very distinct and the proportion of spring wood to summer wood is very variable. In general appearance the wood in transverse section is very like that of *Cedroxylon transiens*, Gothan. There are about fifty rings represented in one of the specimens. Occasional tangential series of resin canals are present, sometimes obviously produced in relation to wounding, but in other instances they appear to have arisen independently. Abnormally large resin canals



TEXT-FIG. 4. *Protopiceoxylon Wordii*, sp. nov. A, detail of a transverse section through the pith with a group of scleroides (thick walls are shown dotted). Brit. Mus., V. 19062. B, radial section of wood to show the pitting on the radial walls of the wood-parenchyma. C, diagrammatic sketch of an instance in which the pitting in the field consists of two double pits. Brit. Mus., V. 19059. Magnification, A, B. and C $\times 350$.

are sometimes found (Pl. XV, Fig. 7), similar to those of *Protopiceoxylon extinctum*, Gothan, from King Charles Land (7, p. 33, Fig. 17), and, as in the latter, the cells surrounding the canals are pitted with small simple pits. These cells are tabular in shape; they measure about 50μ vertically and, with respect to the axis of the canal, 11μ radially and 30μ tangentially. The early-formed tracheides in the annual rings have bordered pits arranged in opposite series and usually flattened and contiguous. They only occasionally alternate (cf. Gothan (8), Pl. II, Fig. 4). The diameter of the pits varies from 16μ to 20μ ; the pore is about one-third to one-fourth the diameter of the pit. A torus is visible in some of the bordered pits, and structures which are to be with considerable certainty interpreted as rims of Sanio are quite a conspicuous feature. The late tracheides have a small lumen and bordered pits are present on their tangential walls.

The medullary rays are normally uniseriate: only occasionally are biseriate rays found. The rays are abundant and may occasionally have a resin canal in the centre. They vary from one to thirty-five cells in height, each ray cell being about 18μ high. The wall of the ray cell has numerous pits on the sides in contact with adjacent ray cells (Pl. XV, Fig. 6).

There are from one to four simple pits in the field. These pits have a major diameter of 3μ where the preservation is good, but elsewhere appear slightly larger; they are occasionally double (Text-fig. 4, C). Wood-parenchyma like that found in *Cedroxylon greenlandicum* (p. 243), but without the pitting on the radial walls, is present on the outer surface of the late wood.

Among the woods described by Gothan from the Upper Jurassic or Lower Cretaceous of King Charles Land (7, p. 32, Figs. 16, 17; Pl. I, 2-5), those which he places in the species *Protopiceoxylon extinctum*, Gothan, are very closely related to the specimen described above. In the latter, however, the bordered pits on the tracheides are normally in two rows and are opposite, frequently with rims of Sanio between the pits, and wood-parenchyma is uniformly distributed throughout the specimen along the outer surface of the late wood. Gothan describes the tracheide-pitting as crowded and alternating when two series of pits are present, but figures a radial section in which many of the pits are opposite (8, Pl. II, Fig. 4). In the same figure are structures which suggest the presence of wood-parenchyma; the long narrow element almost exactly in the centre of the figure appears to be divided up by transverse walls (?), one of which appears at the top and the others in the lower half of the figure. It seems advisable, in view of the markedly Abietineous pitting of the tracheides and the constant nature of the xylem parenchyma, to distinguish this wood as a new species. To place this specimen with *Protopiceoxylon extinctum*, Gothan, is to assume that the same species can exhibit enormous variation in the type of tracheide-pitting and in the relative abundance of wood-parenchyma.

Diagnosis: *Protopiceoxylon Wordii*, Walton. Wood with clearly defined annual rings. Pith parenchymatous, with groups of thick-walled cells. Resin canals, vertical and horizontal, present. Tracheides with one or two series of bordered pits: these are normally opposite. Rims of Sanio present. Pits flattened and in contact. Ray cells thick walled with numerous pits on the horizontal and tangential walls and with one to four pits in the field. Medullary rays one to thirty-five cells high, normally uniseriate. Wood-parenchyma at the end of the year's growth.

Locality: At an altitude of 1,500 feet on the west slope of the mountain (610 metres high according to De Geer) which lies immediately south of Balt Glacier, near Cape Dufferin, Stor Fiord, Svalbard.

Horizon: Middle Jurassic.

Protocedroxylon araucarioides, Gothan.

1910. *Protocedroxylon araucarioides*, Gothan, 'Kungl. Svensk. Vetenskapsakad. Handl.', Bd. 45, No. 8, p. 27, Taf. 5, Figs. 3-5, 7-11; Taf. 6, Fig. 1.
 1913. Cf. *Metacedroxylon araucarioides*, Holden, vol. xxvii of this Journal, p. 533.

1915. *Metacedroxylon scoticum*, Holden, 'New Phyt.', vol. xiv, p. 205, Pl. III, Figs. 1-4.
1919. *Protocedroxylon scoticum*, Seward, 'Fossil Plants', vol. iv, p. 237-8.
1919. *Protocedroxylon araucarioides*, Seward, 'Fossil Plants', vol. iv, p. 236.

In the genus *Protocedroxylon*, Gothan, there is a combination of Abietinean and Araucarian characters. Resin canals are absent. The medullary rays are usually uniseriate, although occasionally partly biseriate rays are found. The cells of the rays are moderately thick walled, with two to six pits on the tangential walls and still more on the horizontal walls. The pits in the field are few in number, usually one, sometimes two or three. Bordered pits in one or two series are found on the radial walls of the tracheides and are generally flattened and in contact. When biseriate the pits alternate.

Two species have been described from Jurassic rocks, *P. araucarioides*, Gothan (8, p. 27), from the Esmark Glacier on the north-west side of Ice Fiord, about forty-five miles from the Cape Dufferin locality, and what was described as a separate species by Miss Holden (10, p. 208) under the name *Metacedroxylon scoticum*, from Loth. in the north of Scotland. It will be shown later that there is good evidence for regarding these two species as one and the same.

Among the blocks collected by Mr. Wordie is one in which part of the pith is preserved, and as this has not as yet been described, a short account of its structure may be given. One has but to compare the photograph of the radial section given by Gothan (8, Pl. 5, Fig. 4) with Pl. XV, Fig. 10, to see that this specimen from Cape Dufferin agrees in all essential features of structure with the type of the species. It is to be noted that the bordered pits on the radial walls of the tracheides are flattened and in contact, and that there frequently shows between each of the pits of a single series a dark line separating the pits. This feature is clearly shown at the bottom left-hand corner of Gothan's photograph, and also in several places in our Fig. 10. There are also abundant tyloses in the lumen of practically every tracheide. The pitting of the ray cells is identical. The only detail in which the Cape Dufferin specimen differs from that from the Esmark Glacier is in the apparent absence of pitting on the tangential walls of the tracheides of the late wood. This in itself cannot be considered as an objection to referring our specimen to Gothan's species. Considering the relation of this specimen to that described by Miss Holden (10, p. 205) we find that the only distinct difference is the absence of partially biseriate medullary rays in the former. This cannot be regarded as a specific distinction, for if the tangential section figured by Miss Holden (10, Pl. III, Fig. 2) is examined, it will be seen that no biseriate rays are represented. Like the Cape Dufferin specimen, that from Scotland has no pits on the tangential walls of the tracheides of the late wood.

It seems, therefore, that one is completely justified in placing all three specimens in the same species, namely, *Protocedroxylon araucarioides*, Gothan. The generic name *Metacedroxylon* (9, p. 539) is not adopted for reasons that have already been stated by Professor Seward (15, p. 238). All the specimens of *Protocedroxylon araucarioides* that have been described possess dark lines between the pits (see above). This feature evidently represents an original structure in the wood and may possibly represent rims of Sanio in a modified or reduced form.

The specimens referred by Miss Holden (9, p. 538) to *Metacedroxylon araucarioides*, Gothan, are possibly of that species, but the published figures do not inspire complete confidence in her identification. Her conclusion (9, p. 208), that the plants from the Corallian of Loth belong to the same 'life-province' as those from West Spitzbergen receives substantial support in the proof of the identity of *Metacedroxylon scoticum*, Holden, and *Protocedroxylon araucarioides*, Gothan.

SUMMARY.

The following species of fossil woods from West Greenland are described :

Cupressinoxylon diskoense, sp. nov. Cretaceous or Tertiary.

Cupressinoxylon, cf. *vectense*, Barber. Cretaceous.

Cedroxylon greenlandicum, sp. nov. ? Tertiary. A well-preserved specimen with the pith almost intact.

The following species from West Spitzbergen, Svalbard, are described :

Protopiceoxylon Wordii, sp. nov. Middle Jurassic. Well-preserved fragments, with a small portion of the pith represented in one of them.

Protocedroxylon araucarioides, Gothan. Middle Jurassic. Well-preserved specimen with half of the pith preserved. The structure of the pith is described for the first time.

The specific identity of *Metacedroxylon scoticum*, Holden, from the Jurassic of Scotland, and *Protocedroxylon araucarioides*, Gothan, from West Spitzbergen, is established.

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EXPLANATION OF PLATE XV.

Illustrating Mr. J. Walton's paper on some Fossil Woods of Mesozoic and Tertiary Age from the Arctic Zone.

Cedroxylon greenlandicum, sp. nov.

Fig. 1. Transverse section of stem. The disturbance in the curvature of the annual rings at the bottom left-hand corner is related to the presence of the vascular supply to a branch. Natural size. Hare Island, north-west of Disko Island, West Greenland. ?Tertiary. British Museum, No. V. 19050.

Fig. 2. Part of the same section more highly magnified to show the pith. The large lacunae are due to mineral structures. No resin canals are present in this part of the section. Cells with dark inclusions are visible in the pith. Magnification, 36 diams.

Cupressinoxylon, cf. *rectense*, Barber.

Fig. 3. Transverse section of a Teredo-bored fragment. The annual rings are visible at the bottom of the figure. The large lacunae are Teredo-borings; some have inclusions of fine sedimentary material. Natural size. Kük, north side of the Nügssuak Peninsula, West Greenland. Cretaceous. British Museum, No. V. 19053.

Cupressinoxylon diskoense, sp. nov.

Fig. 4. Radial section through the secondary wood. Resiniferous parenchyma is present on the left. Biseriate opposite pitting may be seen near the top right-hand corner. Part of a medullary ray is represented at the bottom. Magnified 134 diams. Skansen, south-east coast of Disko Island, West Greenland. ? Cretaceous. British Museum, No. V. 19058.

Protopiceoxylon Wordii, sp. nov.

Fig. 5. Radial section of the secondary wood, showing the characteristic tracheide-pitting. The difference in width of the spring and summer elements is clearly illustrated. The wood-parenchyma which is normally present on the outer surface of the summer wood does not show up in this photograph. Magnified 130 diams. Locality near Cape Dufferin, Stor Fiord, West Spitzbergen, Svalbard. Upper Jurassic. British Museum, No. V. 19060.

Fig. 6. Radial section of the same specimen to show the much-pitted vertical and horizontal walls of the ray cells. The pits on the radial walls appear as one or two small, lighter areas in each field. Magnified 340 diams. British Museum, No. V. 19059.

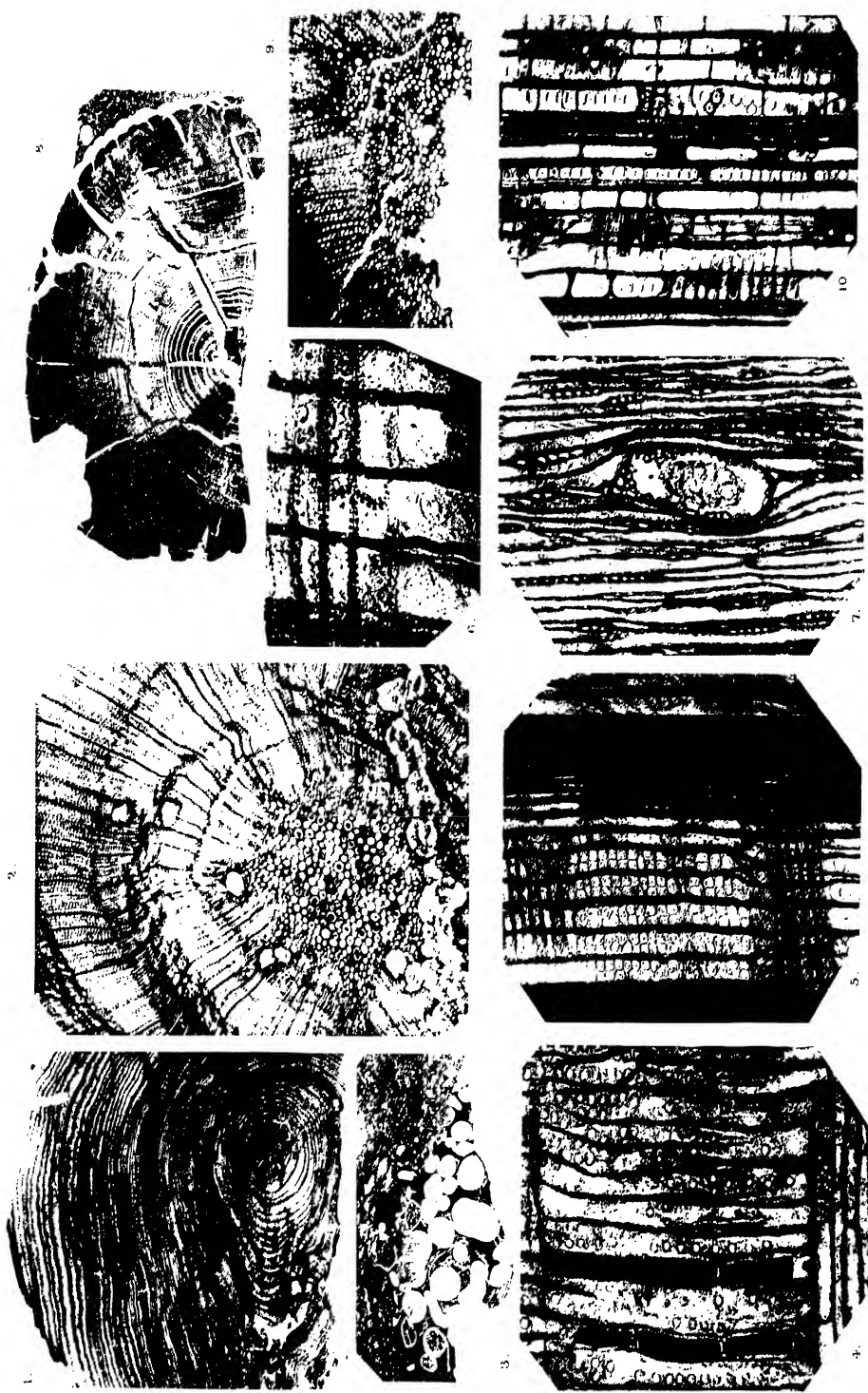
Fig. 7. Tangential section from the same specimen. The typically uniseriate rays are shown, and in the middle an 'abnormal' ray with an included resin canal is represented. Magnified 133 diams. British Museum, No. V. 19051.

Protocedroxylon araucarioides, Gothan.

Fig. 8. Transverse section of a stem (or branch). What appear as cracks in the section are in reality veins of colourless mineral matter. Magnified 158 diams. Locality near Cape Dufferin, Stor Fiord, West Spitzbergen, Svalbard. Upper Jurassic. British Museum, No. V. 19063.

Fig. 9. Part of the same section at greater magnification. Some of the cells of the pith have dark-coloured inclusions, possibly representing resin. Near the top right-hand corner a sinus in the secondary wood indicates the presence of a leaf-trace cut near to its point of departure from the perimedullary region. Magnified 54 diams.

Fig. 10. Radial section cut from the same specimen, showing the abundant tyloses in the tracheides and the nature of the tracheide-pitting. To the left there is a very narrow tracheide of the summer wood with correspondingly small, bordered pits. Magnified 134 diams. British Museum, No. V. 19064.



WALTON — FOSSIL WOOD FROM THE ARCTIC ZONE.

The Relationship between the Phytophthorae associated with the Bud-rot Diseases of Palms.

BY

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JOHNSTONE (12) regarded all cases in which a rot of the terminal bud of the coco-nut palm was involved as due to one disease, viz. 'Bud-rot', the cause of which he ascribed to *Bacillus cili* or an organism indistinguishable from it. His statement that 'the terminal bud will become a soft putrid mass only in the case of bud-rot' is not supported by the observations of later investigators. Nowell (18) has shown that in the final stage of the 'red-ring' disease caused by the nematode *Aphelenchus cocophilus*, there ensues a bacterial soft rot of the tender tissues forming the apex of the stem, as also of the similar tissues at the base of young inflorescences. Petch (31) also records the decay of the terminal bud in the final stage of the root disease caused by *Fomes lucidus*. Ashby (1) reports having seen in Jamaica large trees dying off with symptoms like those of the Cuban bud-rot. 'There were occasional cases in the same spots where trees had been recorded as dying ten years earlier. They could be interpreted as cases of premature senescence under unfavourable external conditions.' Sharples and Lambourne (31) describe an outbreak of 'bud-rot' in Malaya, concerning which they state: 'The badly affected field was inundated twice a day by tidal water and the trees were very backward in consequence. There was little doubt that the primary cause of the trouble in this case was the daily inundations.'

The signs of 'bud-rot' in its incipency as described by Johnstone (12) are: '(1) the falling of immature nuts, (2) the staining of the opening flower spikes, partly or wholly to a rich chocolate brown, and (3) the dying and bending over of the middle undeveloped leaves'. On Pl. IV he shows a diseased tree with pendent older leaves and apparently normal young leaves and central spike. Coco-nut palms are now known to exhibit similar symptoms in red-ring disease, root disease, severe drought, and other ill-defined causes. Consequently, it appears inadvisable to classify all cases of diseased palms exhibiting such symptoms under the common name of 'bud-rot'.

The final stage in the death of a coco-nut palm, no matter what may be the cause of its moribund condition, is usually a rotting of the terminal bud. The rotting of the terminal bud is accompanied by very prominent symptoms. The central unfolded leaves wither and turn brown, and may be pulled out of their sheaths easily by hand. The leaves, so removed, terminate in a putrid, soft, brown, malodorous mass and the bud tissues, instead of being pure white and of firm consistency, are semi-liquid and emit a very disagreeable odour. In an advanced stage the rot includes the whole of the tender tissues at the apex of the stem, and stops only when the woody portion of the stem is reached. The destruction of the terminal bud necessarily leads to the death of the tree, as this is the only vegetative bud produced on the coco-nut palm. The term 'bud-rot' well describes this condition, which has been reported from practically every country in which the coco-nut palm is cultivated.

As 'bud-rot' is always fatal, its occurrence in epidemic form entails great financial loss. The wide distribution of *Cocos nucifera* and its economic importance has led to close studies of the cause of the rotting of its terminal bud by numerous workers. A study of the extensive literature dealing with this phenomenon indicates that it is not necessarily due to the activities of any one specific parasitic organism, nor to the combined effect of any definite set of conditions. In short, the rotting of the terminal bud is not symptomatic of any one disease, but occurs as a result of a number of separate and distinct causes.

It would appear advisable to distinguish between those diseases in which the rotting of the terminal bud is a result of a primary infection of the bud tissues by a specific organism, and those in which the rotting of the bud is secondary and consequent to the tree being brought to a moribund condition through other causes. The name 'bud-rot' should be reserved for such diseases as are caused primarily by an infection by a specific organism in the region of the bud which results in a rot of the tender non-fibrous tissues at the apex of the stem, including the meristematic tissues of the bud, the other organs of the tree being healthy at the time of infection. Direct infection and destruction of the bud tissues result in the death of the central spike of young unfolded leaves before any of the recognizable symptoms occur amongst the older leaves of the crown. Such symptoms give the clearest indication of a primary infection of the bud. In the field the nearest approach to such a specific bud-rot is seen in those cases with which a species of *Phytophthora* (*P. palmivora*) is usually associated. This type of bud-rot is more or less characterized by the occurrence of a decay of the terminal bud before the outer leaves are visibly affected. Bacteria are invariably present in the rotting tissues, but the experimental evidence indicates clearly that their occurrence is of a secondary nature and that they have followed the primary attack by the fungus. McRae (17)

has afforded conclusive evidence that *P. palmivora* can attack mature palms and cause 'bud-rot' without the palms being wounded previous to infection, and has demonstrated that this fungus is the primary factor which has to be controlled in 'bud-rot' epidemics in India. A similar 'bud-rot' has also been produced by infection of unwounded palms with *Phytophthora Faberi* by Reinking (26) in the Philippines, and by Tucker (35) in Porto Rico.

In those cases in which the rotting of the terminal bud is preceded by the drooping and wilting of the older leaves, the fall of immature nuts, and the rotting of the fruiting branches, or other distinctive symptoms unassociated with an infection of the bud tissues, the rot of the terminal bud has been ascribed in many cases to the attack of various strains of bacteria. Experimental evidence offered by investigators as to the ability of different bacterial strains to cause a primary bud-rot of coco-nut palms has met with considerable criticism. Definite proof that the disease can be caused by bacteria alone, unassisted by wounds or other conditions unfavourable to the palm, is still awaited. If a specific bacterial bud-rot of the coco-nut palm exists, from the evidence at present available it appears doubtful if the true causative agent has been isolated.

Nowell (19) has suggested the use of the term 'coco-nut wilt' for certain cases of which the symptoms have a strong resemblance to those described by Johnstone, viz. the rotting of the terminal bud preceded by a drooping of the older leaves; and there appears to be good reason for its general adoption. Its use would avoid the implications which the more general term 'bud-rot' involves and would avoid confusion. To practical agriculturists, diseases bearing the same common name are regarded as identical, due to the same causes and requiring the same treatment. To them a primary or true 'bud-rot' is the same as a secondary 'bud-rot' or 'wilt' so long as the name 'bud-rot' is applied to both types of disease. There is no more reason for describing those cases, for which the term 'wilt' has been suggested, as 'bud-rot' than there is for applying the term 'bud-rot' to undoubted cases of red-ring or *Fomes lucidus*.

The treatment usually recommended for 'bud-rot' (used in its widest sense) is the immediate destruction of the crown by fire. With true bud-rot caused by the attacks of parasitic organisms in or near the bud, the burning of the crown destroys the infectious material in the bud and so reduces the risk of further spread. But the destruction of the crown of a tree of which the bud has rotted consequent to its reduction to a moribund condition by causes other than a direct attack on the bud tissues, i. e. wilt, does not necessarily mean that the risk of further spread of the disease is in any way reduced. The destruction of the crown may be necessary on the grounds of general sanitation, but its value in preventing further spread may be nil. The prompt and systematic destruction of palms affected by

true bud-rot, that caused by *P. palmivora* in particular, has proved very effective in preventing the further spread of the disease (17), but the cutting out of trees affected by wilt has in some instances no visible effect on the steady march of the disease (19).

It is possible that more than one specific disease will be included at present under the name 'wilt'; in which case it will be necessary to apply distinctive names to them as they become differentiated. The use of the term 'wilt' at present would help to distinguish an ill-understood disease condition from one of which both cause and treatment are better known. The effective treatment of true bud-rot consists essentially of the immediate destruction of the crowns of infected palms, whereas for wilt some further measure is likely to prove necessary, the measure depending upon the ascertained cause of the condition. Sharples (29) has adduced reasons for a change of policy respecting the drastic cutting out and burning of all palms suffering or suspected to be suffering from 'bud-rot'. Under the term 'bud-rot' he has included cases of 'wilt'. The destruction of palms suffering from true bud-rot is absolutely essential, though such treatment may not be necessary for wilt. The changes of policy required are the use of a term such as 'wilt' for the diseased condition in which the rotting of the bud is a secondary symptom, the study of the causes resulting in this condition, and the planning of suitable means of prevention and treatment, unhampered by conclusive results obtained from the study of true bud-rot.

P. palmivora, Butl.

In 1906 Butler (5) described a bud-rot of Palmyrah and coco-nut palms in India which he attributed to a species of *Pythium*, viz. *P. palmivorum*, Butl. Later this species was transferred to the genus *Phytophthora* as *P. palmivora*. The investigations of Shaw and Sundararaman (32) and of McRae (17) have confirmed Butler's conclusions, and have demonstrated that the bud-rot of palms on the east coast of India is due solely to the activities of this fungus, which is capable of penetrating the uninjured bud tissues. In Jamaica Ashby (1) isolated from bud-rot of coco-nut palms a species of *Phytophthora*, which was identified in 1920 by Butler as *P. palmivora*. Reinking (25), in 1919, stated that the cause of bud-rot in the Philippines was *P. Faberi*. The same name has been used by Tucker (35) for the species of *Phytophthora* which he isolated from bud-rot in Porto Rico.

On the publication of Reinking's investigations, Butler (6) drew attention to the possible identity of *P. Faberi* and *P. palmivora*, and later (7) stated: 'From a careful study of Reinking's figures and description I am of opinion that this fungus is morphologically identical with my *P. palmivora*.'

Reinking (26), after a careful study of the cross-infection possibilities and the physiological and morphological characteristics of the fungus

strains from coco-nut and cacao in the Philippines, concluded that coco-nut bud-rot and cacao pod-rot are caused by the same species of Phycomycete, which he identified as *P. Faberi*. Tucker (35) compared the measurements of conidia and chlamydospores of his coco-nut *Phytophthora* with those obtained by Rosenbaum (27) and by Reinking for *P. Faberi*. His measurements of conidia corresponded closely with those of Rosenbaum and of Reinking, but his chlamydospores were somewhat smaller. He believed the difference in chlamydospore measurements to be insufficient to warrant the separation of the Porto Rico isolation as a new species. Leonian (14), on the basis primarily of their physiological reactions, groups *P. Faberi* and *P. palmivora* together with other strains under the name *P. omnivora*. This is of interest in that the fungus now commonly known as *P. Faberi* was originally referred to *P. omnivora*, de Bary, by Massee (15). Ashby's comparison between *P. palmivora* from coco-nut and *P. Faberi* from cacao in the West Indies showed that the macroscopic growth and microscopic characters of the mycelium, the size and shape of the sporangia, and the size of chlamydospores of the two fungi were all very much alike, though minor differences were observed. Unlike Reinking, he found biological differences; and in view of these differences, together with certain results obtained with mixed cultures, he maintained *P. Faberi* and *P. palmivora* as distinct species.

The writer is unaware of any detailed comparative study of the morphological characters of the virulent parasitic form of *P. palmivora* as it occurs in India and the usual strain of *P. Faberi* common on cacao. Most investigators have compared a strain isolated from coco-nut with *P. Faberi* from cacao, but not with *P. palmivora* from India, where it was named. Ashby's coco-nut strain was identified by Butler as his *P. palmivora*, but whether this identification was made after a careful comparative study is not evident. *P. Faberi* is known to have a very wide host range, and it would not be surprising that under suitable conditions it should also include the coco-nut, and produce symptoms similar to its allied *P. palmivora*. Undoubtedly, there is considerable similarity between the morphological characters of *P. palmivora* and *P. Faberi*, but owing to the difficulties, to be stated later, in determining species in the absence of oospores, some doubt must remain as to the morphological identity of these two fungi until *P. Faberi* is carefully compared with an authentic strain of *P. palmivora*, preferably from India, where it causes severe epidemics of bud-rot.

Sharples (30) has stated: 'The conclusion has now been reached that the fungus named *P. palmivora* by Dr. Butler, and that isolated from bud-rot in the Philippines and considered by Dr. Reinking as *P. Faberi*, are practically identical, so that there is a common cause for "bud-rot" in both Eastern and Western Hemispheres'. If this conclusion is maintained, then

the name *P. Faberi* for the organism associated with canker and pod-rot of cacao, and by which it is generally known, must be abandoned in preference to the earlier name *P. palmivora*, unless Leonian's suggested use of *P. omnivora* is adopted. This procedure, in view of our present knowledge of the genus *Phytophthora*, is to be deprecated until the morphological identity of the two fungi has been indubitably established. Though biological differences have little value in nomenclature, they are often of great importance in economic biology, and no useful purpose can be served by abandoning a well-known name until the systematic position of the fungus has been very definitely established.

FIELD OBSERVATIONS.

Bud-rot of coco-nuts was first recorded for Ceylon by Petch (20) in 1906. The symptoms described by him indicate indubitably that the disease was a true bud-rot, but as bacteria only were found in the rotting apical tissue, he attributed the cause to bacteria. Since then, bud-rot has occurred sporadically, and has on no occasion become epidemic. In 1924 a species of *Phytophthora* was isolated for the first time from coco-nut bud-rot in Ceylon; since then the same species of *Phytophthora* has been isolated on several separate occasions. The morphological characters of this species are indistinguishable from those of *P. Faberi* from cacao. In this respect the observations in Ceylon agree with the results obtained in the Philippines and Porto Rico. It should, however, be borne in mind that, although the name *P. palmivora* is used throughout this paper for the strain from coco-nut, no direct comparison has been made with *P. palmivora* from India.

P. Faberi is an exceedingly common parasite in Ceylon. It occurs throughout the cacao and rubber districts, where it causes considerable losses annually to these crops. It is also known as a parasite on other host plants. It is, therefore, surprising that epidemics of coco-nut bud-rot have not been experienced, if the fungus of bud-rot is identical with *P. Faberi*, as coco-nuts are grown extensively in both the cacao and rubber districts. Similar facts have been recorded for the Federated Malay States (31). There *P. Faberi* occurs as a parasite of *Hevea*, yet bud-rot has never assumed epidemic form except in the one small instance referred to previously in this paper. Ashby (2) also records that in Grenada, although pod-rot and canker of cacao occur, bud-rots of the coco-nut are rare. That climatic conditions should be unfavourable for the well-being of this fungus on coco-nut in Ceylon appears very improbable. The slight incidence of coco-nut bud-rot at times when *Hevea* and cacao are severely attacked would indicate that there are, at least, marked biological differences between the *Phytophthora* from coco-nut and that from *Hevea* and cacao. It, therefore, appeared advisable to carry out inoculation experi-

ments to determine whether the *Phytophthora* from cacao would readily infect coco-nut, and vice versa.

INOCULATION EXPERIMENTS.

In describing these experiments the name *P. Faberi* has been retained for the strain isolated from diseased cacao pods, and *P. palmivora* for the strain from coco-nut bud-rot. Both strains grew readily on maize-meal agar and cultures on this medium were invariably used for the inoculation experiments.

On cacao pods. Fresh healthy cacao pods were first washed in a solution of corrosive sublimate, and then with sterile water. They were placed in separate glass dishes containing a little distilled water, to provide a damp atmosphere. Four pods were wounded by cross-hatching a small area of the surface with a sterile knife, and inoculated with mycelium of a young culture of *P. Faberi*. Another four pods were inoculated from the same culture, but without previous wounding. As natural infection of cacao pods usually occurs either at the stalk end or at the tip, these inoculations were made near the middle of the pod in order to avoid confusion with any natural infection that may have previously occurred. The wounded pods showed signs of infection at the point of inoculation in three or four days, but the unwounded pods did not become discoloured till from six to nine days. The characteristic symptoms of the disease occurred on all pods; the discoloration started at the point of inoculation, and spread until the whole pod was involved. Abundant conidia and chlamydospores were produced on the surface. No inoculation failed.

TABLE I.
Inoculation Experiments on Cacao Pods.

Strain used.	No. of Inoculations.	Treatment.	Results.		Remarks.
			Positive.	Negative.	
Cacao	4	Wounded	4	0	Severe, within 4 days
Coco-nut	12	Wounded	1	11	Slight, on 11th day
Areca	4	Wounded	4	0	Slight, within 12 days
Cacao	4	Not wounded	4	0	Severe, within 9 days
Coco-nut	6	Not wounded	0	6	—
Areca	4	Not wounded	0	4	—

Twelve inoculations were similarly made on wounded pods with mycelium from a young culture of *P. palmivora*. Only one inoculation proved successful, and then there was no visible sign of infection till after the eleventh day. The remaining pods remained healthy until the end of the third week, when they became invaded by other organisms. Six inoculations were made on unwounded pods with the same fungus, but none gave positive results. The results are collected in Table I.

On coco-nut seedlings. In these experiments a strain of *P. Faberi* from diseased *Hevea* pods was included.

1. On July 3, 1925, two coco-nut seedlings were inoculated with mycelium from a culture of *P. palmivora*, one with *P. Faberi* from cacao, and another with the same species from *Hevea* pods. Two seedlings were kept as controls. The inoculum was placed low down in the central spike between the youngest leaves without previous wounding. As the weather conditions were dry at the time, the plants were covered with large gas cylinders in order to create a moist atmosphere around the central spike. To allow of this being done, parts of some of the leaf-blades had to be removed, but unnecessary mutilation was avoided. The cylinders were removed fourteen days later, but there was no visible sign of infection, nor did any symptom of bud-rot occur later.

2. Four coco-nut seedlings were inoculated on August 12, 1925, with the same fungi as were used in the previous experiment. This time the plants were wounded with a sterile knife which was thrust horizontally into the plant just above the level of the terminal bud, in such a way that a vertical wound was made on the outer petiole. The inoculum was then pushed into the wound with a sterile needle, so that it reached a position just above the bud as far as could be ascertained. Two plants were similarly wounded and kept as controls. These plants were covered as in the previous experiment until the 4th of September, when the cylinders were removed.

One palm inoculated with *P. palmivora* showed a browning of the youngest leaf at the time the cylinders were removed. The infection, however, did not reach the bud or cause a bud-rot, as evidenced by the production of a new leaf later. On the 5th of October, when this experiment was stopped, the innermost leaf, which had newly expanded, was healthy except for small, brown, rotted patches at the apex. The next outer leaf was badly rotted, only the leaf base and petiole remaining. This leaf was young and unfolded at the time of inoculation. The other palm inoculated with the same fungus was not infected, and a healthy new leaf was unfolded before the experiment was stopped.

When the cylinder was removed from the palm inoculated with *P. Faberi* from cacao the central shoot was dead, and the outer leaves were somewhat yellowish. As the outer leaves failed to regain their normal healthy green colour, and there was no sign of any new growth, the palm was dissected on the 7th of October. This disclosed that the bud tissues were destroyed by a soft brown rot. The fungus was reisolated from the diseased bud tissues.

No sign of any incipient bud-rot was noticed in the palm inoculated with *P. Faberi* from *Hevea*, nor in the palms used as controls.

3. On the 7th of October more seedling palms were inoculated as in Experiment 2, but they were not covered with gas cylinders, as weather conditions were wet at this time.

Both palms inoculated with *P. palmivora* died of bud-rot. The

central shoots wilted and could be easily pulled out by hand by the end of the month. The fungus was reisolated from both shoots so removed. As no further growth was made after the central shoots had been removed, the palms were dissected on the 1st of December. The meristematic tissues at the apex of both shoots were found to be destroyed by a soft brown rot.

TABLE II.
Inoculation Experiments on Coco-nut Seedling.

Experiment No.	Strain used.	No. of Inoculations.	Treatment.	Results.	
				Positive.	Negative.
1	Coco-nut	2	Not wounded	—	2
	Cacao	1	"	—	1
	<i>Hevea</i>	1	"	—	1
	Areca	2	"	—	2
	(Control)	2	"	—	2
2	Coco-nut	2	Wounded	—	2
	Cacao	1	"	1	0
	<i>Hevea</i>	1	"	—	1
	Areca	2	"	—	2
	(Control)	2	"	—	2
3	Coco-nut	2	"	2	—
	Cacao	1	"	1	—
	<i>Hevea</i>	1	"	1	—
	Areca	2	"	2	—
	(Control)	2	"	—	2

The palm inoculated with *P. Faberi* from cacao had, at the time of inoculation, a young leaf just beginning to unfold. On the 19th of October this leaf could easily be pulled out by hand. Its basal end was soft and rotten, and a red-brown discoloration extended for a distance of 3 inches up the leaf, but the remainder was green and apparently healthy. It was suspected that this leaf may have been partly severed during the wounding operations. The discoloured end of the shoot was therefore washed with corrosive sublimate solution and distilled water, and placed in a moist chamber. On examination after two days, abundant sporangia of *P. Faberi* were found. This indicates that though the leaf may have been partly severed, *P. Faberi* played some part in the rotting of the tissues. After removing the central spike the palm was attended to as though a normal plant. By May 24, 1926, no new growth had been made, but the three outer leaves remained green and apparently healthy. The palm was, therefore, dissected in order that the apical tissues could be examined. It was found that at the apex of the stem there was an irregular cavity with a blackened wall, but below this the tissues were white and hard. It appeared that the meristematic tissues had been destroyed, but the rot had not progressed any great distance down the stem. This plant is considered to have died of bud-rot, though the characters of the rot were somewhat abnormal.

The other palm inoculated with *P. Faberi* from *Hevea* exhibited a discoloration of the central unfolded leaf and a yellowing of the outer

leaves on the 3rd of November. On the 7th of December the plant was dead with withered leaves. The bud tissues were destroyed as with bud-rot.

No disease symptoms were observed in the control palms. They grew normally, and periodically put out new leaves.

The results of these experiments with seedlings are given in Table II. In recording the results on palms, only inoculations which resulted in a rot of the terminal bud are classified as positive.

Reinking's experiments (26) showed that on coco-nut '*Phytophthora Faberi*, Maubl., from cacao may produce disease by invading directly the young injured leaves, but that infection takes place more readily through injuries'. With the *Phytophthora* from coco-nut bud-rot he was able to cause slight or medium rot of cacao pods. He summarizes his extensive inoculation experiments with these strains as follows (25): 'The consistent similarity in the attack of various hosts by both strains of the fungus shows that in this respect there is a constant likeness. In no case, except probably with the cacao fruit, was there any difference noted in the virulence of the two. Both are omnivorous, capable of attacking a large number of different hosts.'

Ashby (2) found that *P. palmivora* set up an extensive rot of unwounded tissue in the heart of coco-nut palm seedlings, while *P. Faberi* from cacao caused only a restricted infection at wounds. All attempts to infect cacao pods with the coco-nut *Phytophthora* were unsuccessful.

The results of the Ceylon inoculation experiments agree with Ashby's results as regards the infection of cacao. But the occurrence of a definite rotting of the terminal bud of wounded palms infected with *Phytophthora* from cacao indicates that the infection resulting from this strain is not so restricted as Ashby's experiments indicate.

Sharpley and Lamborne (31) have made certain criticisms of the stab method on coco-nut seedlings as proof of the parasitism of any fungus. They were able to cause the primary symptom of bud-rot, viz. a diseased condition of the central leaves, with different strains of bacteria, *Thielavia* sp., *Mucor* sp., and *Phytophthora Faberi*. Only one definite case of bud-rot, as shown by a rotting of the bud tissues, was noted throughout their inoculations, and that was obtained after inoculation with *P. Faberi*.

The object of the present experiments was not to demonstrate the parasitism of *P. palmivora* on coco-nut, as that has already been conclusively proved by McRae, but to make a comparison of the virulence of the coco-nut and cacao strains. The experiments on coco-nut seedlings show that when the palms are wounded and artificial inoculations are made, the strain from cacao will produce a rot of the bud tissues almost as readily as that from coco-nut.

As the investigations had to be stopped before experiments with unwounded palms had been carried out, it remains undetermined whether the strains are equally virulent on unwounded palms. General field observations

indicate that, at least in Ceylon, *P. Faberi* from cacao and *Hevea* are not very virulent on coco-nut, otherwise the comparative freedom from bud-rot of palms growing in the neighbourhood of badly infected *Hevea* and cacao is difficult of explanation. The epidemics of bud-rot in India indicate that *P. palmivora* is a virulent parasite. If, then, the coco-nut *Phytophthora* in Ceylon is identical with *P. palmivora* in India, why are there not periodic epidemics of bud-rot when conditions are favourable? On the other hand, if the Ceylon coco-nut *Phytophthora* is identical with the *Phytophthora Faberi* from cacao, then this species is not very virulent on coco-nut and there are obvious biological differences between the two strains.

OOSPORES.

The presence of oospores in pure cultures of *P. Faberi* and *P. palmivora* has not yet been recorded in nature or on any culture medium.

Ashby (3) first observed the presence of oospores and sexual bodies in mixed cultures of *P. Faberi* and *P. palmivora* isolated from cacao and coco-nut respectively in Jamaica, in 1920. He obtained similar oospores and sexual bodies when the cacao fungus was grown in mixed culture with *P. palmivora* from cotton bolls and with *P. parasitica* from coco-nut and castor. *P. parasitica* produces oospores in pure culture, but they can be distinguished by their size from those produced in mixed cultures of *P. Faberi* and *P. palmivora*. The oospores of *P. parasitica* as well as the '*Faberi*' oospores occurred when *P. parasitica* was grown in mixed culture with *P. Faberi*. The presence of oospores in mixed culture of *P. Faberi* and *P. palmivora* suggested that these may be strains of one heterothallic species, but their presence in mixed cultures of *P. Faberi* and *P. parasitica* cannot be explained on this basis, as *P. parasitica*, owing to its formation of distinctive oospores in pure culture, must be regarded as a distinct species. Ashby, therefore, concluded that 'the absence of oospores both in pure and mixed cultures of this species (*P. palmivora*), and some growth differences, as well as its inability to infect cacao pods, distinguish it from *P. Faberi*'.

Later, the writer (11), after experiments with mixed cultures of seven Ceylon strains of *Phytophthora* morphologically similar to *P. Faberi* from cacao, was able to divide them into two distinct groups, according to their behaviour in mixed culture. One, termed the cacao group, consisted of strains from cacao, papaw fruit, and papaw stem; the other, or rubber group, consisted of strains from rubber, *Odontadenia*, *Dendrobium*, and breadfruit. Oospores were not formed when two strains from any one group were grown in mixed culture; but when the culture contained a strain from each group, oospores similar to those described by Ashby were produced. These results suggested that the strains belonged to one heterothallic species.

As Ashby had obtained oospores by growing the West Indian strains from cacao and coco-nut together in mixed culture, a similar experiment was made with the corresponding Ceylon strains, but without success. Moreover, oospores were not formed when the Ceylon coco-nut strain was grown in mixed culture with the other members of the cacao group. When, however, the coco-nut strain was grown together with the strain from *Odontadenia*, belonging to the rubber group, sexual bodies and oospores, similar to those previously found in Ceylon mixed cultures and described by Ashby for *P. Faberi*, were found in abundance.

The oogonia were spherical, yellow or yellow-brown, thick-walled, and measured $28-36\ \mu$ in diameter. The amphigynous antheridia were hyaline, thin-walled, and varied in length from 12 to $16\ \mu$ and in width from 14 to $18\ \mu$. The oospores were spherical, smooth, hyaline, and varied in diameter from 18 to $30\ \mu$; the mean of fifty measurements being $23.8 \pm 0.263\ \mu$, which agrees closely with the mean measurement of fifty oospores from a mixed culture of the cacao and *Odontadenia* strains, viz. $23.78 \pm 0.148\ \mu$. Ashby gives the measurements of $23.1-23.6\ \mu$ as the range of the mean diameter, and $17.8-28.6\ \mu$ as the range of variation for oospores of *P. Faberi*.

This result definitely indicates heterothallism in *P. Faberi*. If this interpretation is refused, the fact that '*Faberi*' oospores are produced in mixed cultures containing the *Odontadenia* strain and either the strains from cacao or coco-nut, whereas they are not produced in mixed cultures of the two last-named fungi, indicates that the *Odontadenia* strain is *P. Faberi* and the others are a different species. The conclusion that the cacao fungus in Ceylon is not *P. Faberi* is a *reductio ad absurdum*.

Ashby's cross between *P. Faberi* and *P. parasitica* certainly appears to be of the nature of a hybrid. The fact that *P. Faberi* will hybridize with *P. parasitica* cannot, however, be used as evidence against the existence of heterothallism in *P. Faberi*. The two things are quite separate. Ashby's facts may be simply explained on a sexual basis, on the assumption that his cacao strain is female and coco-nut strain is male and supplies the stimulus for sexual reproduction. Then a mixed culture of his cacao and coco-nut strains will contain the male and female sex and stimulus necessary for the production of sexual organs. Similar requisites are brought together when the cacao strain is grown in mixed culture with *P. parasitica*. In this case *P. parasitica* supplies the male element and any necessary stimulus, and the cacao strain provides the female organs; consequently, the oospores produced on the *P. Faberi* thallus are distinct from those on the *P. parasitica* thallus. In that case the coco-nut strain is not necessarily a species distinct from *P. Faberi* from cacao, but may be the complementary thallus of a heterothallic species.

The present experiments indicate that the Ceylon strains from cacao and coco-nut are of the same sex, whereas Ashby's strains from these hosts

are of opposite sex. It would appear probable, then, that if Ashby's strains were grown in mixed culture with the corresponding Ceylon strains, i. e. the cacao with the cacao, oospores would probably occur in one or other of the mixtures. If, as might be expected, oospores occurred in the mixed cacao strains or in the mixed coco-nut strains the question of heterothallism in this species would be definitely proved, as strains having similar morphological characters and causing a disease with similar symptoms on one host plant could not possibly be regarded as distinct species.

REMARKS.

With the removal from the evidence of mixed cultures, Ashby's arguments for the separation of *P. Faberi* and *P. palmivora* become weak. To a systematist, a species consists of individuals with similar morphological characters. Though the *Phytophthora* from coco-nut may differ from the *Phytophthora* from cacao in certain minor characters, these differences are such that they have very little value for taxonomic purposes. Physiological characters, such as are shown by parasitism, though useful for the separation of distinct strains, are not yet regarded as adequate criteria for the differentiation of species.

In the genus *Phytophthora* the most reliable criterion for the separation of species is the size and morphology of the sexual reproductive bodies. The absence of sexual organs makes the determination of a species a very difficult matter, particularly as certain species characterized by their oospores fail to produce them when grown under different conditions. The determination may be made still more difficult by the non-production of chlamydospores. This is well illustrated by the behaviour of *P. Meadii*, McRae, which causes a disease of *Hevea* in South India and elsewhere. McRae (16) at first suspected this species to be *P. Faberi*, but by a careful study of parallel cultures he was able to distinguish them. McRae states that 'the main differences then between the two fungi are that the oospore of *P. Faberi* almost completely fills the oogonial wall, and that no antheridium is present, while in *P. Meadii* the oospore lies loose within the oogonial wall, and a persistent antheridium is invariably present'. The bodies which McRae described as oogonia of *P. Faberi* are now generally recognized to be chlamydospores. In South India *P. Meadii* does not form chlamydospores; but McRae records that a culture sent to Pusa produced resting conidia soon after its arrival, and continued to do so in subsequent sub-cultures. 'The resting conidia also come fairly close to *P. Faberi* in size.' In Burma (9) a disease occurs on *Hevea* similar to that in South India, but the fungus associated with it does not produce oospores, though it does form chlamydospores normally. McRae (16) considers the Burma and South Indian *Phytophthorae* to be identical, viz. *P. Meadii*. From the literature there appears to be little by which the Burma fungus

could be distinguished from *P. Faberi* on purely morphological grounds. If, then, McRae's determination of the Burma fungus is correct, and there appears no obvious reason to dispute it on physiological grounds, it becomes evident that the identity of a strain of *P. Meadii* which does not produce oospores is likely to be confused with *P. Faberi*.

Owing to the great range of variation in the size of chlamydospores and conidia of most species of *Phytophthora*, the identification by these characters in the absence of oospores is difficult, and in some cases impossible if the method of expressing the measurements commonly used in mycology is adopted, viz. the range of variation. Rosenbaum (27) in his studies of the genus *Phytophthora* used the more refined statistical method. In his '*Faberi*' group, characterized by the antheridium being entirely unknown, or its relation to the oogonium not yet determined, he includes two species, *P. Faberi* and *P. jatrophae*, which he separates by the mean diameters of their chlamydospores. It appears to have been overlooked that Rutgers (28) has figured antheridia, oogonia, and oospores of *P. jatrophae*, the antheridium being definitely amphigynous. The removal of this species from Rosenbaum's '*Faberi*' group leaves *P. Faberi* as the only species concerning which antheridia are unknown in nature or in pure culture, though no doubt a search through the literature of this genus would disclose other named species which would belong to this group, e.g. *P. cinnamomi*, Rands (24). Lafferty and Pethybridge (13) list seven other named species, in which the mode of development of the sexual organs is not fully known, or in which these organs have not yet been found. This list includes two, *P. fici*, Rau, and *P. citri*, Rau, which were provisionally named in 1915, and concerning which nothing has been further published, and two, *P. Faberi* and *P. Theobromae*, known to be synonymous. The separation of species belonging to this group by chlamydospore measurements is open to objections on the grounds of (1) the uncertainty of chlamydospore production, and (2) the variability of chlamydospore measurements.

As regards the uncertainty of chlamydospore production, Butler (5), concerning *P. palmivora* on Palmyrah, states that resting conidia could not always be found, but sometimes they could be, and then were present in very large numbers; they were either absent or rare at certain seasons and in some years. McRae (16) states that the same experience has been found with this fungus on coco-nut palms in South India. 'Resting conidia are seldom seen, but early this year (1918) they were found in very large numbers indeed...' The behaviour of *P. Meadii*, as regards chlamydospore production, has already been noted. The writer has found that different strains believed to belong to the species *P. Faberi* vary considerably as to the ratio in which chlamydospores are produced relative to sporangia.

To prove the variability in the size of chlamydospores of a species such as *P. Faberi*, one has but to compare the measurements given by various authors. Tucker (85), concerning this *Phytophthora* from coco-nut, states: 'Considerable discrepancy may be noted between the diameters of chlamydospores which were grown on potato-dextrose agar, and of those that were produced on cotton meal, the most populous class advancing from 31.5μ to 33.49μ for the former, to 37.5μ to 39.49μ for the latter medium, on which the measurements obtained are somewhat smaller than those recorded for *P. Faberi* by both Rosenbaum and Reinking. Delacroix and Maublanc describe "oospores" (chlamydospores) 45μ in diameter. The difference in chlamydospore measurements is believed to be insufficient to warrant the separation of the Porto Rico isolation as a new species.' The size of sporangia, as well as of chlamydospores, is influenced by other conditions such as moisture, light, and temperature, as well as by differences in the chemical composition of the substratum on which they are grown. Plastic characters, which vary with external conditions, are of little value for taxonomic purposes; their value decreases as their plasticity increases. The difficulty concerning the plasticity of certain characters may be overcome, no doubt, by culturing the fungi under definite standard conditions. Unfortunately, in the experience of the writer, species of *Phytophthora* do not grow well on synthetic media. They grow well on a substratum containing vegetable extracts, as bean agar and maize agar, but it is wellnigh impossible to standardize such media owing to the variable composition of suitable vegetable material.

In view of our present knowledge of the morphological characters of those species of *Phytophthora* which do not normally produce sexual organs, it seems to the economic mycologist inadvisable to abandon at present the well-known name of *P. Faberi* for the species parasitic on cacao, in favour of the name *P. palmivora*, although there appear to be some reasons for regarding the two as synonymous. When the characters of all the species of this group have been carefully studied, and a sound method of classification has been devised, and the parasitic habits of the individual species determined, it may then be necessary to alter permanently the name by which certain species are known. Until then, the abandonment of certain well-known names in preference of others, which appear more correct to the systematist at the moment, is more of a hindrance than a help to the economic mycologist. The retention of the name *P. Faberi* for the strain occurring on cacao, and *P. palmivora* for that occurring on palms, can lead to little confusion so long as the possibility of their synonymy is borne in mind. To the economist there is much in favour of their distinction, as, at least as regards Ceylon, there appears, from field observations, little risk of bud-rot through growing coco-nuts in the neighbourhood of cacao infected with canker or pod-rot.

Phytophthora Arecae, Colem.

In 1910 Coleman (8) described a disease of the areca palm which is characterized by the fall and rotting of the immature fruits. Though the disease is usually confined to the nuts, the tops of the trees occasionally become infected. An infection in the crown, other than on the fruits, can only be made out after it is fairly well advanced and the growing point itself has become infected. Then the growing point is badly decayed and the tree dies. Coleman ascertained that 'infection of the tree-top may take place, on the one hand, directly from the diseased bunch by means of mycelium growing down through the stalk, and thence up to the growing point, and, on the other hand, by means of independent infection of the leaf sheaths surrounding the growing point of the tree. Actual examination of dying tree-tops shows that the first method is the more usual, but that the second method is also to be met with occasionally.' He also demonstrated by experiment that infection of the top could occur through the leaf sheaths by means of spores which have germinated on the outer surface. This disease, therefore, though primarily a fruit disease, may under certain circumstances take the form of a 'bud-rot'.

The causal organism associated with this disease of areca was first identified by Sydow and Butler (34) in 1907, as *Phytophthora omnivora*, de Bary. As Massee (15) had also referred the fungus associated with the pod-disease of cacao to the same species, Coleman made a comparative study of the *Phytophthorae* from areca and cacao. The cacao *Phytophthora* was isolated from Ceylon material. As a result of this study he concluded that, although the sporangia and the zoospores of the areca and cacao fungi are practically identical in size, the two fungi could be distinguished by their oospores. Concerning this he states: 'As far as I am aware there is no record of the finding of antheridia in the cacao *Phytophthora*, and all the evidence at hand goes to indicate that the oospores, at least in the majority of cases, develop parthenogenetically. In this respect the cacao *Phytophthora* contrasts strongly with the form from areca, where antheridia seem to be universally present, and where the remains of them could be found attached to the oogonial wall long after the oospores had been fully formed, and were quite mature'. He therefore proposed the name *Phytophthora omnivora* var. *Arecae* for the areca fungus and *P. Theobromae* for the cacao fungus. Maublanc, however, had previously published the name *P. Faberi*, and it is by this name that the cacao fungus is generally known. The variety *Arecae* of the species *P. omnivora* in recent literature ranks as a distinct species, viz. *P. arecae*.

The spherical bodies of *P. Faberi*, which Coleman regarded as parthenogenetic oospores, are now regarded as asexual resting spores or chlamydospores. Rosenbaum (27) reported an abundance of oospores, the

sexual bodies being of the amphigynous type, in his cultures of *P. arecae*, but did not record the presence of chlamydospores. The absence of chlamydospores and the presence of oospores are the morphological characters by which *P. arecae* can be distinguished from *P. Faberi*.

In 1924 Sundararaman and Ramakrishnan (33) reported a disease of coco-nuts in certain parts of Malabar, characterized by the dropping of young and nearly mature nuts in large numbers. *P. arecae* was found to be the cause of this disease; inoculation experiments showed that the coco-nut fungus produced the characteristic fruit-rot of areca-nuts, and the fungus from areca palms was equally parasitic on coco-nuts. A noticeable fact was that the disease occurred only on coco-nut palms growing in association with areca palms. Oospores were not found on the coco-nut fungus, and there is no mention of chlamydospores. Petch (22) in 1917, recorded a similar disease of coco-nuts in Ceylon, with which a species of *Phytophthora* was associated. The fungus, however, was not identified nor described, nor was any mention made of association of the diseased palms with arecas.

Butler (5) states that in India a bud-rot of areca palms is caused by *P. palmivora*, though areca-nuts are less liable to attack than coco-nuts. *P. palmivora* is reported by Ashby (1) to cause a rotting of coco-nut husks and the fall of full-grown but immature nuts.

There are thus two fungi, *P. arecae* and *P. palmivora*, known to cause bud-rot of areca palms and nut-fall of coco-nuts.

OBSERVATIONS IN CEYLON.

In 1923 some areca palms in the Karawanella district were affected by a fruit disease resembling that described by Coleman. The disease was characterized by a fall and rotting of immature nuts. The first sign of infection was the presence of dark-green patches of variable size near the base of the fruit. These patches had a water-soaked appearance which clearly distinguished them from the clear-green colour of the healthy nut. When infected nuts were placed in a damp chamber, a copious, white, fluffy mycelium, bearing *Phytophthora* sporangia and chlamydospores, developed from the water-soaked areas. A similar mycelium was found on the surface of some of the freshly fallen nuts, but on others, which had reached a more advanced stage of decay, the mycelium was not evident. On these the base or the whole of the pericarp was brown and shrivelled. On placing the seed or portions of the seed of these fruits under sterile conditions in Roux tubes, a similar mycelium was obtained. Following the fall of the fruits, the fruiting branch dried up and became black and withered.

In October, 1924, a similar disease occurred on areca palms in the Kegalle district. Again there was a fall of immature nuts, but the most notable feature of this attack was the withering and drying out of the young nuts, together with the blackening of the fruiting branch. A species of

Phytophthora, similar to that isolated the previous year, was obtained from the infected fruits. In December a number of the infected palms died, owing to a rot of the terminal bud. An examination of the crowns of the dying trees showed that the disease had progressed upwards from the infected fruit branches, until the bud tissues became involved. This was evidenced by the outer leaves dying first, and the central spike being the last part of the crown to wither. A dissection of the crown of a dying tree demonstrated that the primary infection had not occurred in the region of the terminal bud, but that the decay had progressed upwards from a diseased fruit branch. A strain of *Phytophthora*, similar to that obtained from the nuts, was isolated from the bud tissues.

The progress of the disease from fruits and branches to the terminal bud was similar to that described by Coleman, as the usual course of infection with *P. arecae* in India. Moreover, at the time the areca fruits were affected in Kegalle, 'a nut-fall' of half-grown, immature nuts from neighbouring coco-nut trees was reported. The fallen coco-nuts showed large brown discoloured areas near the base of the fruits similar to those reported by Petch and by Sundararaman and Ramakrishnan. Attempts to isolate the fungus from diseased coco-nuts were unsuccessful, so that no direct evidence was obtained that the cause of the coco-nut nut-fall was due to the same organism which caused the fruit-rot of areca, but the investigations of Sundararaman and Ramakrishnan into the relationship between the fruit-falls of areca and coco-nut are suggestive.

THE FUNGUS.

The strains of *Phytophthora* isolated from the rotting fruits and from the bud tissues of areca grew readily on maize-meal agar. The strains were indistinguishable in culture, so that one strain only need be described. The strain selected was one isolated from the bud tissues.

The sporangia were prominently papillate like those of *P. Faberi*. They measured $48-70 \times 30-48 \mu$. No oospores were found, but in old cultures spherical bodies resembling chlamydospores, though not distinctly yellow, occurred. These, however, were not produced abundantly, as is the case with *P. Faberi*. They measured $24-48 \mu$ in diameter. The absence of oospores and presence of chlamydospores led to the assumption that the strain was not *P. arecae*. Further consideration, however, showed that the absence of oospores in culture—they were absent also from fresh material—was no criterion, as the literature on the subject indicates that there is much variability and uncertainty in the production of oospores in culture, and the conditions which actually determine the production of sexual bodies in *Phytophthora* species are unknown.

The size and shape of the sporangia, together with the presence of chlamydospores, suggested that the strain might be *P. Faberi* or *P. palmi-*

vora, although the field characters certainly indicated *P. arecae*. Rosenbaum gives $47.9 \pm 0.374 \mu$ and $48.5 \pm 0.198 \mu$ as the mean lengths of conidia of *P. arecae* and *P. Faberi* respectively, and $30.05 \pm 0.186 \mu$ and $32 \pm 0.159 \mu$ as their respective mean widths. In these respects the conidia of these two species are not markedly different. As Coleman separated these species principally because of the difference between the oospores, the chlamydospores of *P. Faberi* being considered as oospores, it would appear that the strain isolated from areca in Ceylon is not *P. arecae*, but must be regarded from its morphological characters as *P. Faberi*, *P. palmivora*, or a new allied species, unless it should be proved that *P. arecae* produces chlamydospores under certain circumstances.

It seemed possible that the behaviour of the areca fungus in mixed cultures with various strains of *P. Faberi* might offer some indication of its identity. Consequently, different strains from areca were grown in mixed culture with strains of *P. Faberi* from cacao, papaw, *Hevea*, *Dendrobium*, *Odontadenia*, and breadfruit, and with *P. palmivora* from coco-nut. Oospores, however, were not found in any of these mixed cultures. The fact that every strain of *P. Faberi* used in these experiments has produced oospores of the '*Faberi*' type when grown in mixed culture with other strains, and the absence of oospores from all cultures when the areca strain was used suggest that the latter is not a heterothallic strain of *P. Faberi*, but possibly belongs to a species not allied to *P. Faberi*. The value of this negative evidence is somewhat doubtful, as the result of growing an authentic strain of *P. arecae* with *P. Faberi* is not known. Whether the former species would cross with *P. Faberi*, as does *P. parasitica*, has not been determined.

In view of the uncertainty as to the determination of the areca fungus it appeared advisable to carry out cross-inoculation experiments with the Ceylon strains from cacao, coco-nut, and areca.

INOCULATION EXPERIMENTS.

On cacao. The results of the inoculation experiments in which the cacao and coco-nut strains were used have already been described. The results obtained from the following experiments with the areca fungus are included in Table I, so that they may be readily compared with those obtained from the cacao and coco-nut strains.

Eight cacao pods were prepared as in the previous experiments. Four were inoculated through wounds made with a sterile knife with material from young cultures of the areca *Phytophthora* grown on maize-meal agar; the remainder were inoculated without being previously wounded. All the wounded pods became discoloured at the places of inoculation in ten to twelve days and the infection spread slowly. In no case did the whole

pod become involved. None of the unwounded pods showed any sign of infection after twenty-one days, when the experiment was stopped.

The areca fungus was therefore not so virulent as *P. Faberi* on cacao.

On coco-nut seedlings. Inoculation experiments on coco-nut seedlings were made with the areca fungus at the same time and in the same way as those with the cacao and coco-nut strains already described and recorded in Table II.

1. Two unwounded palms were inoculated on July 3, 1925, with the areca fungus. One remained healthy and showed no visible sign of infection. The other showed a slight infection on the youngest leaf (a bifid leaf, usual with seedlings) when this became expanded. A narrow strip of tissue extending half-way across the leaf blade had rotted and fallen out, as though a piece had been roughly cut out. The fungus was not reisolated, but the position and appearance of this lesion indicated that this was a direct result of inoculation. This result is not recorded as positive in Table II as a rot of the terminal bud had not been produced.

2. Of the two seedlings inoculated on August 12, 1925, one appeared to be infected at the time the gas cylinder was removed. The visible part of the youngest leaf appeared blackened and dead. By September 9 this leaf had elongated and it was evident that the infection had been restricted to the distal end of the leaf, and had not reached the bud tissues. Later, the leaf became fully expanded, and, except for the destroyed tip, there was no sign of any further infection on this palm. The other palm remained healthy and showed no visible result of the inoculation.

3. Both plants inoculated on October 7, 1925, with the areca fungus died as a result of a rotting of the terminal bud. By October 23 a young unfolded leaf of one palm had begun to dry up and turn brown. The wilting leaf was pulled out easily by hand; the end was rotten and the *Phytophthora* was recovered from it. By the beginning of December the whole plant was dead, all the leaves were withered, and the bud tissues were rotted. The progress of the disease on the other palm was similar. By the beginning of November the youngest of the outer leaves was yellowing and the central, youngest leaf was discoloured and apparently dead. By the end of the month the plant was dead and the terminal bud rotted.

This fungus is, therefore, quite as virulent on coco-nut palms as the strains from coco-nut bud-rot and cacao.

On areca fruits. For these experiments two strains of the areca fungus were used (A from fruits and B from bud tissue), two strains from coco-nut bud-rot, one from cacao, and one from *Hevea* fruit-rot. The fruits were first washed with corrosive sublimate solution and distilled water, and glass boxes were used as moist chambers. Wounds were made as before with a sterile knife. Five fruits were used as controls, three of which were wounded. These remained healthy till the experiments terminated.

Four wounded fruits and six unwounded fruits were inoculated with the areca fungus A. The wounded fruits all showed signs of infection within three days and a copious mycelium enveloped the fruits within five days. Of the unwounded inoculations only one gave a negative result; the other five produced copious mycelium and sporangia within eight days.

Of the inoculations made with strain B of the areca fungus, those on wounded fruits (four) gave positive results, but only two inoculations out of five proved successful on unwounded fruits. Where positive results were obtained, infections were as rapid as those of strain A.

TABLE III.

Inoculation Experiments on Areca Fruits.

Strain used.	No. of Inoculations.	Treatment.	Results.		Remarks.
			Positive.	Negative.	
Areca A	4	Wounded	4	—	Severe
Areca B	4	"	4	—	Severe
Coco-nut A	5	"	—	5	
Coco-nut B	5	"	—	5	
Cacao	5	"	1	4	Slight
<i>Hevea</i>	2	"	—	2	
(Control)	3	"	—	3	
Areca A	6	Not wounded	5	1	Severe
Areca B	5	"	2	3	Severe
Coco-nut A	4	"	—	4	
Coco-nut B	4	"	—	4	
Cacao	4	"	—	4	
<i>Hevea</i>	2	"	—	2	
(Control)	2	"	—	2	

Five inoculations were made with each strain of the coco-nut fungus on wounded areca fruits and four with each on unwounded fruits. No infection occurred on any fruit.

On wounded fruits, five inoculations were made with the cacao fungus and two with the strain from *Hevea*. Four unwounded fruits were inoculated with the cacao fungus and two with the *Hevea* strain. Of these only one gave a positive result, and that occurred on a wounded fruit inoculated with the cacao fungus.

It was evident from these experiments, summarized in Table III, that only the areca fungus was virulent on areca fruits; and the results suggest that this strain is truly *P. arecae*.

On areca palm seedlings. For these experiments also two strains of the areca fungus, two from coco-nut bud-rot, one from cacao, and one from *Hevea* were used. As both strains from areca and from coco-nut gave identical results, the strains have not been distinguished in Table IV, which summarizes the results.

I. Inoculations made on April 18, 1925. Two palm seedlings about six months old were used for each strain. The inoculum obtained from young pure culture on maize-meal agar was pushed down as far as possible into

the centre of the crown with a blunt sterile needle without wounding the palms. The plants were then covered with wide lamp chimneys, the open ends of which were closed with cotton-wool. The object of so covering the plants was to create a damp atmosphere favourable to the fungus. The chimneys were removed on the following day. No palm at any time showed any sign of infection. This was possibly due to unfavourable climatic conditions and to the short time the palms were covered. There was a small rainfall on most of the days during which the palms were under observation, but the palms were in the shelter of the plant house.

TABLE IV.

Inoculation Experiments on Areca Seedlings.

<i>Experiment No.</i>	<i>Strain used.</i>	<i>No. of Inoculations.</i>	<i>Treatment.</i>	<i>Results.</i>	
				<i>Positive.</i>	<i>Negative.</i>
1	Areca	4	Not wounded	—	4
	Coco-nut	4	"	—	4
	Cacao	2	"	—	2
	Hevea	2	"	—	2
	(Control)	2	"	—	2
2	Areca	4	Wounded	4	—
	Coco-nut	4	"	—	4
	Cacao	2	"	—	2
	Hevea	2	"	1	1
	(Control)	4	"	—	4
3	Areca	4	"	4	—
	Coco-nut	4	"	2	2
	Cacao	2	"	2	—
	Hevea	2	"	1	1
	(Control)	4	"	—	4

2. Inoculations made on July 4, 1925. In this experiment, after washing with corrosive sublimate solution, the palms were wounded in the same manner as were the coco-nut seedlings in the experiments already described. Two palms were inoculated with each strain, and four, after wounding, were kept as controls. The plants were covered as before with lamp chimneys, which were not removed till the seventh day, as climatic conditions proved unfavourable.

After two or three days the youngest and innermost leaves of two of the control plants were observed to be wilting, and they could be easily pulled out by hand. The bases of these leaves, however, showed no sign of a soft-rot characteristic of bud-rot. Investigations showed that these leaves had become severed or partially severed near the base during the process of wounding with the sterile knife. Later, these palms produced new and healthy leaves and normal growth was continued. A similar wilting of the central leaf was observed amongst the inoculated palms. In some cases the base of the leaf was somewhat rotted at the time of removal, and the fungus was reisolated from the base of the petiole. It was evident, however, that the mere wilting of the central leaves could not be interpreted as

a first indication of infection, and that a surer sign of infection was the failure of the palm to produce new leaves. Palms which failed to produce new leaves after a suitable interval of time and which showed other signs of disease, such as a wilting of the older leaves, were dissected so that the terminal bud could be examined. In every case in which a dissection was made as a result of non-formation of new leaves, the terminal bud was found to be destroyed, and in many cases the fungus was recovered from the decaying bud tissues. Only those inoculations which resulted in the destruction of the bud tissues are recorded as positive in Table IV.

The four palms inoculated with the areca fungus were killed within fourteen days. The rot spread through all the leaf bases so that the whole of the aerial part of the seedling collapsed. The fungus was reisolated without difficulty.

The four palms inoculated with the coco-nut fungus and the two with the cacao fungus gave like results. The central shoots wilted and collapsed within fourteen days, but the outer leaves remained healthy and green. When the experiment was stopped in the middle of September, these palms had formed new leaves which had expanded normally and showed no indication of any infection.

One of the palms infected with *P. Faberi* from *Hevea* pods died as a result of bud-rot, but the other recovered after the central shoot wilted. At the beginning of September, all the leaves of the palm which became infected had turned brown, and a dissection showed that the bud was destroyed. The other palm had formed a new leaf and growth was proceeding normally.

3. Inoculations made on October 7, 1925. Two palm seedlings about nine months old were inoculated with each strain as in Experiment 2. As the weather conditions were more suitable for inoculation experiments with *Phytophthorae*, the palms were covered for twenty-four hours only. Four similarly wounded palms were used as controls. These grew normally and produced new leaves even when the central spike was severed during the wounding process.

The four palms inoculated with the areca fungus died of bud-rot within ten days and the fungus was recovered from the decaying bud tissues. Two palms were inoculated with the coco-nut strain A, and two with the strain B. Of these one palm of each pair died and the other recovered after the central shoot had wilted. The progress of the disease in the palm inoculated with strain A was almost as rapid as with the palms inoculated with the areca fungus, the complete withering of the outer leaves occurring in fourteen days. The progress of the disease in the palm inoculated with strain B was much slower, and it was not until after one month that the leaves completely withered. The fungus was recovered from both palms affected by bud-rot.

Both palms inoculated with the cacao fungus failed to produce new leaves after the withering of the central spike. The outer leaves, however, remained green for a long time (until the beginning of December), so that the progress of the decay was slow, like that resulting from inoculation with coco-nut strain B. The fungus was reisolated from both palms. One palm inoculated with the *Phytophthora* from *Hevea* died within fourteen days, and the fungus was reisolated from the rotting bud tissues. The other palm inoculated with this strain recovered and produced new leaves after the central spike had wilted.

On coco-nut fruits. 1. The same strains from areca, coco-nut, and cacao were used as in the previous experiment. The nuts (4 to 5 inches long) were first washed with corrosive sublimate solution and distilled water. Two inoculations were made with each strain from pure culture on unwounded fruits, and two on wounded fruits on March 11, 1925; four nuts were kept as controls. The fruits were placed in moist chambers and kept in the laboratory.

TABLE V.
Inoculation Experiments on Coco-nut Fruits.

Experiment No.	Strain used.	No. of Inoculations.	Treatment.	Results.	
				Positive.	Negative.
1	Areca	2	Wounded	2	—
	Coco-nut	2	"	2	—
	Cacao	2	"	2	—
	(Control)	2	"	—	2
	Areca	2	Not wounded	—	2
	Coco-nut	2	"	—	2
	Cacao	2	"	—	2
	(Control)	2	"	—	2
	Areca	2	"	—	2
	Coco-nut	2	"	2	—
2	Cacao	2	"	—	2
	(Control)	2	"	—	2

No infection occurred on any of the unwounded fruits. After three days the wounded fruits inoculated with the areca and coco-nut strains became discoloured, and the discoloured zones extended rapidly. Discolorations were first noticed on the fruits inoculated with the cacao strain on the fourth day, and these spread relatively slowly. Sporangia were produced in abundance on all fruits except those inoculated with the areca fungus, on which mycelium was abundant but sporangia were scarce.

2. On March 24, unwounded fruits were inoculated with material obtained from the wounded fruits of the previous experiment. On April 1 the fruits inoculated with the two coco-nut strains became discoloured at the inoculated places. The discoloration spread until the whole of the fruits were involved; sporangia were produced in abundance on the discoloured areca. No other strain gave positive results.

The results of the above experiments are given in Table V. All

strains proved capable of setting up a rot of the pericarp of coco-nut fruits when infection was made through a wound. Only the coco-nut strains were able to infect unwounded fruits.

CONCLUSION.

The above experiments disclose certain biological differences between the strains used. The markedly greater virulence of the areca strain when inoculated on areca fruits, coupled with symptoms observed on areca in the field, suggests strongly that the areca strain is Coleman's *P. arecae*. This conclusion is suggested by the results of Coleman's cross-inoculation experiments. He inoculated three small cacao beans¹ with zoospore suspensions from pure cultures of the areca *Phytophthora*. Of these one remained quite unaffected; one, the smallest, showed the signs of typical brown-rot and on microscopic examination revealed *Phytophthora* mycelium in the tissues, but no sporangia on the surface and no formation of oospores. The third also became decayed, but apparently as a result of some slight injury and saprophytic attack, as a microscopic examination revealed no sign of *Phytophthora* either on the surface or in the tissues. On the other hand, inoculation of areca nuts from pure cultures of the cacao *Phytophthora* were even less successful.

If it is accepted that the Ceylon areca strain is *P. arecae*, it becomes evident that *P. arecae* under certain conditions produces chlamydospores, though normally in India it does not. In this respect it resembles *P. Meadii*, which produces chlamydospores in Burma but not as a rule in South India.

The morphological differences between *P. arecae*, *P. Meadii*, *P. Faberi*, and *P. palmivora*, in the absence of oospores, are not strongly marked. Consequently, the accurate determination of these species in the absence of oospores is difficult, if not impossible in our present state of knowledge, if the determination is made on morphological characters alone. Oospores of *P. arecae* and *P. Meadii* are known in nature, and in pure culture and the two species can be distinguished by the characters of the sexual bodies. Oospores of *P. Faberi* and *P. palmivora* are not known either in nature or in pure culture. The similarity of the known morphological characters of these fungi (including the absence of oospores) has led to the conclusion that they are one and the same species. It should follow, then, that any strain of *P. Meadii* (such as the Burma one) or of *P. arecae* (such as the Ceylon one) of which oospores are unknown either in nature or pure culture should be classified morphologically with the '*Faberi-palmivora*' species. Unless this view is taken the synonymy of *P. Faberi* and *P. palmivora* cannot be maintained on morphological grounds alone.

¹ From the context it appears that Coleman used cacao seeds and not entire pods for his inoculation experiments.

SUMMARY.

1. The general adoption of a term such as 'wilt' is urged for those ill-understood diseases of palms, in which the rotting of the terminal bud is preceded by the drooping and wilting of older leaves.

The name 'bud-rot' should be applied only to those diseases in which the rot of the bud is the result of a primary infection in or near the bud tissues.

2. The effective treatment of bud-rot consists essentially in the immediate destruction of the crowns of infected palms, whereas for 'wilt' some further measure is likely to prove necessary, the measure depending upon the ascertained cause of the condition.

3. The occurrence is recorded of a bud-rot of coco-nut palms with which a species of *Phytophthora*, possibly *P. palmivora*, is associated.

4. The occurrence of a species of *Phytophthora* believed to be *P. arecae* is reported as the cause of a fruit-fall and rot of the terminal bud of areca palms in Ceylon.

5. Oospores of the Ceylon strain of *P. arecae* have not been found in nature nor in pure cultures. Chlamydospores occurred in culture.

6. The similarity of the morphological characters (in the absence of oospores) of *P. Faberi*, *P. palmivora*, *P. arecae*, and *P. Meadii* is commented upon. The only safe criteria for the determination of species in the genus *Phytophthora* are the size and characters of the sexual organs.

7. Cross-inoculation experiments with Ceylon strains from cacao, coco-nut, and areca are described, and biological differences between these strains are noted.

8. Experiments were carried out with mixed cultures of strains of *P. Faberi* and the strains from coco-nut and areca. No oospores were obtained when the areca strain was grown in mixed culture with any of the *P. Faberi* strains. Oospores were obtained in abundance when the coco-nut fungus was grown with a strain of *P. Faberi* from *Odontadenia*.

9. The Ceylon strains from coco-nut and cacao are of the same sex, whereas in the West Indies the strains from these hosts are of opposite sex.

10. The question of the synonymy of *P. Faberi* and *P. palmivora* is discussed. It is considered inadvisable at present to abandon, in favour of *P. palmivora*, the name *P. Faberi* for the strain parasitic on cacao, although there appears to be strong morphological reasons for regarding the two as synonymous. If undue emphasis is placed on the plastic morphological characters of *Phytophthora* strains of which oospores are unknown, the Burma strain of *P. Meadii* and the Ceylon strain of *P. arecae* must either be regarded as belonging to the species *P. palmivora* or new species must be created to accommodate them.

11. A detailed study of the morphological and biological characters of the strains belonging to the 'Faberi' group of Rosenbaum's or Lafferty and Pethybridge's classifications of the genus *Phytophthora* is required.

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Observations on the Development of a Pink Colour in Lignified Tissues by the Chloramine Reaction.

BY

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DURING the progress of a research on the chemical nature of the cell membrane in plant tissues, use was made of a reaction for protein in the cell-wall.

The tissue concerned, in the form of sections, was submitted to the action of chlorine gas for a prolonged period, and subsequently treated with sodium phosphate and potassium iodide solutions (1). The presence of protein in the cell-wall was indicated by the liberation of iodine from potassium iodide. Lignified tissues gave diverse results by this method, the colour developed showing varying shades of yellow to brown, and brown to pink.

In many cases a pink colour appeared, and its mode of occurrence was investigated fully. The production of these diverse colorations by lignified tissues shows that the condition of the lignin or its cellulose base varies in different structures. The pink colour is probably associated closely with the chemical nature of these substances. It may be either a result of the chemical condition of the cellulose base, or it may indicate the existence of a definite compound produced in some way from the lignin.

The work was begun in 1924, and since then about 150 species of plants have been examined, including leaves, petioles, aerial stems, underground stems, hypocotyls, and roots.

The pink colour was developed in xylem and sclerenchyma, but not in any other tissue. It occurred in the xylem of 53 per cent. of the aerial stems, 15 per cent. of the petioles, 15 per cent. of the underground stems, and 22 per cent. of the roots, though, in the last three categories, the pink colour appeared only in organs which had developed secondary xylem.

In no case did it appear in the xylem of the leaves examined. The age of the stem was the first factor to be considered. To this end the hypocotyls of several plants were examined, but the pink colour never developed in the xylem. In *Acer pseudoplatanus* and *Pyrus malus* it appeared in the older stems, although it was absent in the xylem of the hypocotyls.

Apparently, the compound causing the pink coloration is not developed in very young structures, the cell-walls of the tissues of which are chiefly parenchymatous.

Young and old aerial stems of the same plant were next examined; the pink colour was more marked in the secondary xylem than in the primary xylem, in many cases appearing only in the xylem of the older stems.

In *Tilia vulgaris* the pink colour was not developed in the secondary xylem even of old stems.

In stems showing secondary thickening the pink colour often appears, when the young stems show a yellow or brown xylem. *Corylus avellana* forms an exception, for this coloration was absent from the primary xylem of the old stem, whereas it was present in that of the young stem. But there are many instances of the pink colour occurring in both young and old stems and in primary and secondary xylem. On the whole, it may be concluded that the appearance of the pink colour is confined to stems of more than one year old where there is any alteration in the colour of the xylem between a young and old stem, but that it is not necessarily a function of age.

On comparing the colours produced in the primary and secondary xylem, it was found that in 22 per cent. of the specimens examined the two tissues differed. Since its occurrence is not constant in the same specimen, but varies with the age and the time of formation of autumn and spring wood, it appears to be due to some substance which is developed when secondary lignified walls are produced.

As a general rule the autumn wood was deeper in colour than the spring wood. This may be due in part to the closer packing of the cells, but the depth of colour was such that this could not be the case entirely. It was particularly noticeable in *Corylus avellana* and *Larix europaea*.

Generally speaking, the pink colour is confined to, or is much deeper in, spring wood than in autumn wood.

However, the actual season of the year does not appear to influence the distribution of the pink colour through the whole of the xylem. Sixty per cent. of the plants examined during the winter months, and 53 per cent. of those examined during the spring and early summer, showed the pink colour.

It was thought that possibly the development of this colour was associated with a definite class of plant, and as it had been observed in many members of the Rosaceae, plants belonging to the Rosales were investigated.

The organs used were mainly aerial stems, and of these 76 per cent. produced the pink colour in either primary or secondary xylem, so that this class of plant exhibits a definite tendency to produce the effect in

question. But in underground stems the colour was not observed, and only in one case was there any pink tinge in the xylem of roots.

The effect produced on sclerenchymatous tissues was considered next. The pink colour did not appear in the cortical sclerenchyma of leaves or underground stems.

Forty-nine per cent. of the aerial stems containing this tissue showed a pink coloration, while 37 per cent. gave a yellow to brown colour, and 14 per cent. remained uncoloured.

Stelar sclerenchyma in leaves produced a pink colour in 57 per cent. of the plants used, and 45 per cent. showed a yellow colour.

Urtica dioica was the only species which showed a pink colour in the stelar sclerenchyma of its underground stem.

This coloration was never produced in roots. Sixty-seven per cent. of the aerial stems showed the pink colour, 23 per cent. yellow to brown, and 10 per cent. were quite uncoloured.

It is concluded, therefore, that the pink coloration is a function of stelar sclerenchyma rather than cortical sclerenchyma, and that it is associated with aerial stems rather than with other organs.

Where the sclerenchyma was uncoloured in the chloramine test, evidently it consisted only of cellulose.

There was little difference between spring and winter coloration, but where this difference was observed, in most cases the pink colour appeared in the sclerenchyma of those plants examined during the winter months (60 per cent.), while in 21 per cent. of the specimens the pink colour did not appear during the winter. So the compound producing this coloration seems to develop chiefly in the winter in sclerenchymatous tissues.

When young and old aerial stems of the same plant were compared, the sclerenchyma was found to remain the same colour, whether pink or brown; while in a few cases there was deepening in colour in the older stems.

In 36 per cent. of the plants examined the sclerenchyma and xylem in the same plant differed markedly in colour. In the majority of these cases the sclerenchyma gave the pink coloration, while part at least of the xylem produced a brown colour, although often it was yellow to brown, only occasionally remaining uncoloured.

This points to the development of the compound responsible for the pink coloration in tissues the function of which is largely mechanical support, and hence possibly it may exercise a hardening or preserving effect upon the cell-wall.

The colour produced in the chloramine test on sclerenchyma was less marked than on the xylem in many instances, though both tissues are lignified; possibly this is due to a lower percentage of lignin and a consequent higher percentage of cellulose in the sclerenchyma than in the xylem.

By comparison of the results by the chloramine reaction with the effects produced by Knaggs's test (2) for oxycellulose, both before and after chlorination, some interesting conclusions were drawn.

This test consists in the treatment of the sections with hydrochloric acid, washing with water, dyeing deeply with benzopurpurin, then acidifying with the same acid, and finally washing in tap-water.

Where oxycellulose is present a blue colour is produced, and where cellulose and hydrocellulose occur the tissues retain the deep crimson of the benzopurpurin.

In the majority of cases, absence of oxycellulose in the original material, resulting in its detection after chlorination, was associated with the pink colour developed in the xylem in the chloramine reaction. Conversely, presence of oxycellulose in the original material, resulting in a more intense reaction after chlorination, was associated with a brown colour in the xylem in the chloramine reaction.

So in these cases it seems apparent that the lignin is associated with a more or less oxidized condition of the cellulose.

Furthermore, this latter condition appears to occur in leaves, petioles, underground stems, roots, herbaceous and seedling stems, where little or no secondary xylem has been developed. This seems to support the hypothesis that the substance responsible for the pink coloration is a more or less necessary constituent of tissues, one of the functions of which is mechanical support, and indicates that this substance is not due to oxidation of the cellulose base.

In cases where the benzopurpurin was not absorbed and only a yellow colour was produced in the cell-wall, the pink colour appeared in the xylem; evidently some substance was present which inhibited the absorption of the benzopurpurin by the tissue concerned. With sclerenchymatous tissues the effects were not so easily interpreted.

In most cases (62 per cent.) inability to absorb benzopurpurin, or a marked absence of oxycellulose, was associated with the development of the pink colour in both cortical and stelar sclerenchyma. Only 26 per cent. of the plants examined possessed sclerenchyma which was capable of absorbing benzopurpurin, and of these 50 per cent. gave a reaction for oxycellulose and no pink colour, while 50 per cent. indicated absence of oxycellulose and no pink colour appeared in the chloramine test. Probably the pink colour is associated with the absence of oxycellulose in the cell-walls of sclerenchyma, and since there is often no absorption of benzopurpurin, as in the xylem, some substance is present which inhibits the cellulose base from absorbing benzopurpurin.

The conditions under which the pink colour was developed having been determined, the chemical nature of the substance producing this effect is now under investigation.

SUMMARY.

1. The chloramine reaction, used primarily to detect protein in the cell-wall, produces either a pink, yellow, or brown colour in lignified tissues.
2. The pink colour appears chiefly in the secondary xylem of aerial stems of more than one year's growth, though it may occur in both young and old stems and in primary and secondary xylem.
3. Autumn wood gives a deeper pink colour than spring wood, but the actual season of the year does not appear to influence markedly its distribution through the whole of the xylem, though possibly it develops more often in winter.
4. In the Rosales the xylem of aerial stems exhibits a definite ability to produce this coloration, but it does not appear in underground stems or roots.
5. It is a function of cortical rather than stelar sclerenchyma, and in these tissues it is more marked in aerial stems than other organs. It is more pronounced in winter. There is no difference of colours in the sclerenchyma of young and old stems.
6. In many cases the sclerenchyma and xylem differ in colour in the same specimen.
7. Generally, where the cellulose base shows the presence of oxycellulose by Knaggs's test, the pink coloration does not appear either in xylem or in sclerenchyma.

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Further Studies of the Brown-rot Fungi.

II. A Contribution to our Knowledge of the Distribution of the Species of *Sclerotinia* causing Brown-rot.

BY

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DURING the last ten years the writer has obtained from various sources abroad a number of specimens and cultures of the species of *Sclerotinia* causing the brown-rot diseases of fruit trees, in order to compare them with the fungi causing similar diseases in this country. The results obtained, though by no means exhaustive, may not be without interest to those who are studying these diseases, which are widespread and take a heavy toll of the world's fruit crop each year.

Since Woronin's paper of 1900 (40) appeared, it has been generally recognized that there are, in Europe, two species of fungi commonly associated with the brown-rot diseases, viz. *Sclerotinia fructigena* and *S. cinerea*. These two species are rife on the Continent, and both are of common occurrence in this country. The status of *S. laxa* has been discussed in a previous paper (36); morphologically and culturally this fungus of the apricot was found to be similar to *S. cinerea*, and there is no clear evidence at present that it is different biologically from the latter. This conclusion is supported by Chabrolin (4), who has made detailed observations on brown-rot of apricots in the Rhone Valley. In the present paper, therefore, *S. laxa* is considered as synonymous with *S. cinerea*. Two other species of *Sclerotinia* (related to *S. fructigena* and *S. cinerea*, but readily distinguished from these by the presence of disjunctors between the conidia) found on cultivated fruits, viz. *S. Mespili* and *S. Cydoniae*, are known on the Continent, and have been recorded for this country (38, 39); they are of minor importance economically, and will not be considered further in this paper.

The biology and parasitism of the two common brown-rot fungi, as related to diseases of fruit trees in this country, have been described in previous articles (31-7), and the chief points only will be recapitulated here.

S. fructigena is the commonest cause of fruit-rot in ripening apples, pears, and quinces, and it is also frequently found on plums and cherries. *S. cinerea* is not only a common cause of fruit-rot, particularly in plums and cherries, but it also infects the flowers, causing 'blossom-wilt', and in plums infects the leaves and young shoots (33, 37).¹

It has been shown (34, 35) that *S. cinerea* is represented in this country by two biologic forms, one which causes a blossom-wilt and canker of apple trees, and another which infects the fruit and flowers of plum and cherry trees.² The apple blossom-wilt fungus *Sclerotinia cinerea* forma *mali* has been studied chiefly in relation to serious outbreaks of the disease in the southern counties of England (31), but it has also been isolated by the writer from apple spurs received from Scotland and from Ireland. *S. cinerea* f. *pruni* is also generally distributed in those parts of the British Isles where plums and cherries are grown.

Another brown-rot fungus, not recorded for this country, is that prevalent in the United States. In the morphology of its ascigerous and conidial fructifications it is clearly closely related to *S. cinerea*, and in American plant-pathological literature it is generally given that name. It was formerly referred to as *S. fructigena*, but recent workers, as Matheny (18), Conel (5), Valleau (29), and Bartram (3), consider this name was given in error, as there is no good evidence that *S. fructigena* has ever been found in America, even in its conidial stage.

A brown-rot fungus discovered in Pennsylvania in 1883 was named by Winter (30) *Ciboria fructicola*; this name, however, according to Ezekiel (11), 'cannot be assigned to any of the species now known, since the description does not differentiate between them'.

In 1917 the present writer (31) pointed out that this common American brown-rot fungus could be distinguished in cultures from the European forms of *S. cinerea*. Its habit of growth on agar media was different, and it produced conidia freely on such media under conditions which completely suppressed conidia production in the European fungus. A difference could also be observed in the mode of branching of the germ tube of the conidium. For convenience this fungus was later (35) referred to as *S. cinerea* f. *americana*. Recently certain American workers have studied this side of the problem. Norton and Ezekiel (22) consider the differences are sufficient to raise the American form to specific rank, and suggest the name *Sclerotinia americana*, (Wormald) Norton and Ezekiel. Roberts and Dunegan (24), however, maintain that this conclusion is untenable, for, although they offer confirmation of the fact that cultural differences are to

¹ Both fungi have been found occasionally on hosts other than those here mentioned; this point will be dealt with in a future article.

² Killian (16, 17) finds that on the Continent the form of *S. cinerea* on acid cherries is biologically different from the form on sweet cherries.

be recognized, they consider them insufficient (in the absence of any evidence that the American form is distinct from *S. cinerea* morphologically, except in the mode of branching of the germ-tube) to warrant the adoption of another name. One argument used by Roberts and Dunegan against the use of a distinguishing name for the American form is that, with successive sub-culturing, this fungus loses one of its characteristic features, that of free sporing. The fact remains, however, that, when freshly isolated, the fungus invariably produces conidia readily on agar plates or slopes; a single conidium isolated on a prune-agar plate will germinate and give rise to mycelium bearing tufts of conidial chains within four or five days.

Ezekiel, in a more recent paper (11), records the occurrence of the European *Sclerotinia cinerea* in certain parts of America, and restates his claim for the use of the name *Sclerotinia americana* for the more common form found in North America. He has made a comparative study of the two fungi and finds that they can readily be distinguished, not only in the more prolific production of conidia by *S. americana*, but also by their habit and rate of growth on various culture media, and in the mode of branching of the germ-tube and of the hyphae. He also considers that in their mode of parasitism the two fungi are not identical, and concludes that 'the species (*S. cinerea* and *S. americana*) thus not only differ morphologically and physiologically, but their effect on the host appears sufficiently distinct to necessitate separate treatment from a pathological view-point'.

Should further investigation confirm Ezekiel's conclusions with regard to their parasitism (and observations made by Barss (2) go in the same direction), then plant pathologists must recognize them as at least different forms, and they must be studied with a view to restricting their distribution to the regions where they are found already.

In the following discussion the present writer adopts the name *Sclerotinia americana*, as proposed by Norton and Ezekiel, when referring to the common brown-rot fungus of North America.

The areas from which cultures or specimens have been received, with an account of the results obtained from comparing and identifying the fungi in pure cultures, are as follows:

The Continent of Europe. The studies of Woronin (40), Aderhold and Ruhland (1), Frank and Krüger (12), Sorauer (26), and many others show that *S. fructigena* and *S. cinerea* are common and very destructive on the Continent. From a comparison of over twenty strains¹ received from Holland, France, Italy, Switzerland, and Russia (either as cultures or as specimens from which cultures were obtained), it is found that the two common forms which occur in Britain are identical with the Continental

¹ By 'strain' is here meant a pure line; any one strain may or may not be distinguishable culturally from others.

species. In culture the various European strains show slight variations among themselves, and the same is true of strains occurring in Britain; thus, with regard to both *S. fructigena* and *S. cinerea*, when grown on prune juice agar, some strains remain colourless, while others develop a brown coloration in varying degrees.

The fungus causing blossom-wilt and brown-rot canker of apple trees in this country, *S. cinerea* f. *mali*, is probably present on the Continent, since Eriksson (10) has described a disease of apple trees in Sweden with similar symptoms. Eriksson refers to the fungus as *S. fructigena*, but from his description it is evidently *S. cinerea*, and probably therefore f. *mali*. The writer had not yet been able to obtain a specimen or culture of *S. cinerea* f. *mali* from abroad.

One culture, received from Dr. Westerdijk of the Centraal-bureau voor Schimmelcultures, Holland, proved to be indistinguishable from *S. americana*. This result was unexpected and inquiries were therefore made as to its origin. Dr. Westerdijk supplied the information that the strain was isolated from an apple (variety Prince Bismarck) in Holland. As this would appear to be the only occasion that this species has been isolated from European specimens its further distribution in Europe is not known. If this fungus is really a native of Europe, and the specimen from which it was isolated was the result of a natural infection in the open, it is surprising that the fungus is not more generally distributed in Europe, for in other parts of the world (mentioned below) in which it has been found it is very prevalent and most destructive.

The United States. From 1915 to 1924 the writer examined in all twenty-one strains isolated from specimens found in the United States; these were supplemented in 1925 by a set of twenty-five cultures received from Prof. H. P. Barss.

From 1915 to 1919 nine strains were obtained from apple, peach, and plum grown in the States of Oregon, Wisconsin, Virginia, New York, and North Carolina. From the study of these it was first realized that the common brown-rot fungus of America was not identical with either *S. fructigena* or *S. cinerea* as found in Europe, and to distinguish it this form was referred to as *S. cinerea* f. *americana*. As mentioned above this form has been raised by Norton and Ezekiel to specific rank as *S. americana*.

In 1920 Dr. W. L. Howard sent, from California, mummied fruits of peach and apricot, and an apricot twig. Isolations from these proved to be identical, not with the typical American form, but with the common form of *S. cinerea* found on stone-fruits in Britain and on the Continent.¹ In the following year further samples of mummied apricots and peaches were

¹ These results, not published at the time, were held over for incorporation in the present paper. In the meantime the same conclusion was arrived at by Ezekiel (11), who published his results in 1924.

received from California ; the fungus present in them was again found to be *S. cinerea* f. *pruni*. About the same time (February, 1921) peach mummies, sent from Kentucky, were examined and the fungus in these, in contrast with the Californian specimens, proved to be *S. americana*. During 1922-4 specimens and cultures received from the States of Washington, New York, Maryland, and Virginia were also *S. americana*, but a culture obtained from California in 1924 again proved to be *S. cinerea* f. *pruni*.

A fungus causing disease in pears, apples, and various stone-fruits in the coastal regions of Oregon, has been studied by Jackson (14), Posey (23), and Barss (2). It was recognized by them as distinct from the common brown-rot fungus of America, and since the latter at that time was generally considered by American plant pathologists to be identical with *S. cinerea*, the Oregon fungus was thought to be a form not previously described, and the name *Sclerotinia oregonensis*, Barss and Posey, was proposed for it. Ezekiel's work (11), however, suggested that *S. oregonensis* might be identical with *S. cinerea* f. *pruni*. Professor Barss therefore sent to the writer a number of his cultures. Six of these (labelled as *S. cinerea*) were seen to be the common American form, *S. americana* ; the rest (referred to as *S. oregonensis*) isolated from various hosts, e.g. pear, apple, plum, peach, apricot, were indistinguishable in cultures from *S. cinerea* f. *pruni*.

The conclusion to be drawn from the evidence available therefore is, that, in the Pacific coastal regions of the United States, *S. cinerea* f. *pruni* (the form of *S. cinerea* found on stone-fruit trees in Europe) is the brown-rot fungus commonly met with, while in the rest of the States *Sclerotinia americana* prevails.

The numerous papers which have been written by American workers on the biology and control of the brown-rot diseases testify to the enormous damage caused by these diseases in the United States. References to the more important of these papers up to 1925 will be found in a recent article by Rudolph (25).

British North America. In 1916 a number of mummied plums and peaches were received from Mr. W. A. McCubbin, who had collected the specimens in Ontario. Isolations were made from these, and the fungus was found to be the common American form, *Sclerotinia americana*. Shortly afterwards Mr. McCubbin found an ascigerous stage on plums and peaches, and cultures prepared from ascospores were also sent over for comparison with the other strains. These again proved to be *S. americana*.

More recently (in September, 1925) plums were received from the Fraser Valley, British Columbia, sent by Mr. J. W. Eastham. The specimens were fruit of that year's crop (not yet mummified when received), and they arrived in a rather pulpy condition. On placing particles of the flesh on prune-agar plates, however, a fungus grew out ; its mode of growth

and production of conidia within a few days soon proclaimed it also to be *S. americana*. In the letter accompanying the specimens Mr. Eastham stated that in Vancouver Island a brown-rot fungus believed to be Barss's *S. oregonensis* occurred. With regard to the damage caused by the two fungi he remarked, 'The rot which has been sent you occasions a certain amount of twig blighting, but we have had no complaints of any serious blossom injury. The *oregonensis* appears to occur chiefly at the southern extremity of Vancouver Island, and its economic effects are almost entirely confined to a very extensive blossom blight of Olivet cherries, though sweet cherries and plums also suffer to a less degree.' Included in Professor Barss's collection of cultures of *S. oregonensis* (referred to above) were two strains isolated from cherries collected in Vancouver Island; these, in common with the other strains included under that name, were found to correspond to *S. cinerea* f. *pruni*, and there can be no doubt, therefore, that this fungus also occurs in Vancouver Island.

The distribution of the two fungi *S. americana* and *S. cinerea* in British North America thus corresponds to that in the United States. The commoner form is *S. americana*, but in the south of Vancouver Island *S. cinerea* is found.

That these fungi are responsible for considerable damage to the stone-fruit crops in British North America is shown by the work of McCubbin (20) and Eastham and Ruhmann (9).

Australia. The fungus generally associated with the brown-rot diseases in Australia has, until recently, been referred to as *Monilia fructigena*. Thus in 1902 McAlpine (19) uses that name in describing brown-rot damage to apricots, peaches, and cherries in Victoria and South Australia. That the organism observed by McAlpine was not, however, *Monilia fructigena*, but a fungus with grey conidial fructifications, is shown by his descriptions, for he writes, 'The rotting of the fruit is the most striking symptom, with the ash-coloured spores produced on the surface', and again, 'Conidia are produced at the surface in such countless numbers as to give the portion attacked an ashy-grey appearance'. Such descriptions suggest that the fungus was either *Sclerotinia cinerea* or *S. americana*.

In 1922 Harrison (13) described 'the perfect stage of a *Sclerotinia* causing brown-rot of fruit' in New South Wales. He remarked that 'the organism is probably *Sclerotinia fructigena*'. This conclusion appears to have been arrived at in error by comparing the fungus with a strain wrongly named. Mr. W. L. Waterhouse later, in a letter to the writer, says, 'Mr. Harrison is now satisfied that the ascigerous strain he has is *S. cinerea*. The culture obtained from America and labelled *S. fructigena* is quite certainly wrongly named.' It is not clear, however, whether any distinction was made between *S. cinerea* and *S. americana*.

Previous to the publication of Harrison's paper Mr. Waterhouse had

sent to the writer, in 1921, mummied fruits of peach, nectarine, and Japanese plum. Cultures isolated from these showed that the fungus present was identical with the American form *S. americana*. In the following year Mr. Waterhouse sent a culture from an apple, and ascosporic cultures from plum and apple. These again proved to be *S. americana*, and cultures isolated from peach twigs sent by Mr. C. C. Brittlebank¹ from Victoria were also *S. americana*.

The brown-rot fungus generally distributed in the fruit-growing regions of Australia would appear therefore to be *S. americana*. The occurrence of either *S. cinerea* or *S. fructigena* in that continent is at present doubtful. Of the latter species Mr. Waterhouse in October, 1922, wrote,² 'We have not yet got it'.

New Zealand. The conidial stage of a brown-rot fungus, referred to at the time as *Sclerotinia fructigena*, was first recorded for New Zealand by Kirk (15) in 1905. An ascigerous stage was found by Cunningham (6) in 1922. In a recent work Cunningham (7) describes the brown-rot diseases in New Zealand, and refers to the fungus causing the damage as *Sclerotinia cinerea*, Schroeter. He quotes, however, *Sclerotinia americana*, Norton and Ezekiel, as a synonym without distinguishing between the two. Cunningham states emphatically that 'In New Zealand *S. fructigena* does not occur'.

In 1921 the writer examined material sent to him from New Zealand by Dr. K. M. Curtis. The specimens included apricot, peach, and plum mummies and peach twigs. Isolations were made from these, and in every case they gave rise to cultures of *S. americana*. This species is probably, therefore, the fungus responsible for the greater part, if not the whole, of the brown-rot damage in New Zealand. There is no evidence at present that either *S. fructigena* or *S. cinerea* (in its restricted sense as used in this paper) is present in that country.

South Africa. Brown-rot diseases have been recorded for South Africa by Doidge (8); the fungus was quoted as *Sclerotinia fructigena*, but no evidence was given as to its identity with that species. Brown-rot is apparently seldom met with in the fruit-growing regions of South Africa, doubtless as a result of the dry climate, and the writer has not yet succeeded in obtaining specimens, although requests have been made to several observers. All agree that the diseases rarely occur and specimens are difficult to obtain. In the article referred to above Dr. Doidge writes, 'Happily South African conditions do not seem favourable to the fungus causing the disease, and it is only in exceptionally wet seasons that it has been noticed as causing any appreciable damage'.

¹ In the letter accompanying his cultures Mr. Brittlebank wrote, 'The Brown Rot is, under certain conditions, one of our worst orchard diseases. A few years ago it took every fruit in an orchard which had at least 25,000 bushel cases of late peaches.'

² In correspondence.

Japan and Manchuria. In 1911 Takahashi published an account of the 'Sclerotinia diseases of Rosaceous fruit trees in Japan'. He describes a cherry disease caused by *S. Kusanoi* and a 'blossom blight of the apple'. The symptoms of the latter bear some resemblance to those of the 'blossom-wilt' of apples in Britain, but the conidia of the fungus are stated to be provided with disjunctors, which thus distinguish it from any form of *S. cinerea*. These two diseases appear to be unrecorded elsewhere.

In that author's *résumé* (28) of his original Japanese paper he writes : ' *Sclerotinia cinerea* occurs in Hokkaido on the blossoms of apricot, *Prunus Mume*, and *P. tormentosa*, and on the fruits of cherry and apricot. *S. fructigena* is quite common in Japan on the fruits of apple and pear. It also appears on the ripe fruits of quince, cherry, apricot, peach, plum, *Prunus Mume*, *P. Pseudo-Cerasus*, grape, and *Elaeagnus macrophylla*'.

The present writer has not yet obtained brown-rot material direct from Japan, but he was able, on the advice of Dr. Takewo Hemmi, to get in touch with Mr. Michiya Miura, the pathologist attached to the Agricultural Experiment Station in South Manchuria, who sent over five cultures of brown-rot fungi labelled :

1. *Monilia fructigena*, peach fruit, Japan.
2. *Monilia fructigena*, pear-fruit, South Manchuria.
3. *Monilia laxa*, cherry, South Manchuria.
4. *Monilia laxa*, Chinese plum, South Manchuria.
5. *Monilia Kenjiana*, n. sp., apple fruit, South Manchuria.

The two strains of *M. fructigena* were similar to the European strains in their general habit of growth on agar plates and on sterilized potato, on the former growing out uniformly to the edge of the plate without producing conidia, and on the latter forming tufts of yellow conidiophores and conidia. The Japanese strain (No. 1) however produced, in several trials, a zone of black sclerotia towards the edge of prune-agar plates, a feature that has not been observed in any other strain of *S. fructigena*.

The two strains named *Monilia laxa* behaved as strains of *S. cinerea* from other sources in not producing conidia on prune-agar plates but developing grey conidial tufts readily on transferring to sterilized potato. The habit of these two strains on prune-agar plates was somewhat different from other strains of *S. cinerea* in growing out more regularly and in producing short scattered aerial hyphae, giving the culture a pilose appearance. There seems to be no reason at present why they should not be included under *S. cinerea*, which does include strains showing slight cultural differences as already mentioned.

The fifth strain is unlike any other strain of the brown-rot fungi studied by the writer; in his hands it has not produced conidia even on sterilized potato.

DISCUSSION.

Until comparatively recently it had been customary to designate as *Monilia* (*Sclerotinia*) *fructigena* any brown-rot fungus met with, but it is now recognized that there are at least four different brown-rot fungi (either species or biologic forms), each of which is responsible for considerable damage to the world's fruit crop. These fungi differ, not only in their habit when grown in pure cultures on prepared media, but also in their mode of parasitism. From the point of view of the plant pathologist (and ultimately that of the fruit-grower also) they must therefore be considered as entities, and their distribution must be studied with a view to guarding against the introduction of one or another into territories where it is not at present found.

So far as the evidence goes it would appear that *Sclerotinia fructigena* is absent from the great fruit-exporting regions of North America, Australia, and New Zealand. In Europe this is the fungus *par excellence* responsible for the rotting of apples and pears as they approach maturity on the tree and in the early stages of storage. It is not uncommon to see, in autumn, the ground under the trees strewn with fruit in various degrees of infection and many of them covered with the ochre-yellow *Monilia* fructifications. Again, apples infected with *S. fructigena* about the time they are put into the store may show no noticeable sign of infection, but after some time they become quite black and leathery, often with no trace of the fungus on the surface but with the flesh permeated with fungal hyphae. This type of 'black-rot' has been studied on the Continent, chiefly by Molz (21), and in this country by Spinks (27). Both attribute this disease to *S. fructigena*, and the writer (32), too, has invariably isolated this fungus from naturally infected black apples¹ except in the case of two apples sent to him from Oregon; the latter were infected with *S. americana*. In countries where *S. americana* is prevalent and *S. fructigena* absent apples appear to suffer little from brown-rot diseases. If, however, *S. fructigena* became established in the three regions mentioned it is conceivable that it would have an adverse effect not only on the growing apple crop but also on the fruit during transport.

In Britain apple blossom-wilt, caused by *S. cinerea* f. *mali*, is at times very destructive to certain varieties of apples, destroying the inflorescences, causing cankers, and killing back branches. There is no evidence that this disease is present in the chief apple-growing countries outside Europe.²

¹ The writer recently obtained through the courtesy of Dr. G. H. Pethybridge a black apple found at Covent Garden in a consignment of apples imported from Russia; particles of the flesh placed on agar plates yielded cultures of *S. fructigena*.

² Ezekiel (11) however finds that two of his strains of *S. cinerea*, isolated from stored apples at Washington, D.C., show certain cultural features in common with f. *mali*.

With the single exception of its discovery in Holland (already referred to) *S. americana* is unknown in Europe. Whether this fungus would be as destructive here as in those countries where it is present already cannot be foretold, but its introduction is most undesirable. The introduction to Europe of certain American parasitic fungi, e.g. American Gooseberry Mildew (*Sphaerotheca mors-uvae*) and the Downy Mildew of the vine (*Plasmopara viticola*), has been disastrous.

Since both *S. cinerea* and *S. americana* are found in the west of North America an opportunity is presented for a careful comparative study of the two, particularly with respect to their parasitism and their morphology. Experimental work in the open with the American fungus cannot be carried out in Europe without running the risk of introducing a new parasite, but this objection does not hold in the western States and in British Columbia, and it is highly probable that further comparative tests and field observations will be carried out now that it is known that both these fungi occur there. This side of the problem has already been approached by Ezekiel (11). Certain morphological points need further elucidation. Hitherto it has not been possible to study fresh material of the ascophores of the two species side by side, for although the ascigerous stage of *S. americana* has been found on many occasions and in great quantity in America, Australia, and New Zealand, this stage of *S. cinerea* has been found very rarely and never under conditions when a direct comparison with fresh ascophores of *S. americana* could be made. Another feature with regard to the morphology of the two species, which might well repay investigation from the systematist's standpoint, is the size of the conidia produced in winter and spring on the fruit, spurs, and shoots infected the previous season. *S. cinerea* f. *pruni* and f. *mali* under these conditions produce conidia considerably smaller than those found on the fruit or flowers during the spring and summer of the year of infection. Whether this also obtains with *S. americana* appears not to be mentioned by American observers. Material examined by the writer, though not yielding conclusive evidence on this point, suggests, however, that there may be a difference here. In this country it has been found (35) that the winter conidia of *S. cinerea* have an average size of about $11.5 \times 8 \mu$, while the summer conidia average about $18 \times 13 \mu$. The dimensions of conidia on mummied fruit received from America were obtained in those cases where the mummies arrived with viable conidia in sufficient numbers for obtaining representative measurements; in the following, 100 conidia were measured in each. The species of fungus isolated in each case, as determined later culturally, is stated.

It will be seen that the Californian specimens (infected with *S. cinerea*) bore conidia of a size corresponding to the winter conidia of *S. cinerea* in England. The conidia on the other specimens, infected with *S. americana*, were appreciably larger both in range and in average size. Whether such

differences are shown on mummies of the two species when examined immediately after removal from the tree can only be determined on the spot.

Fruit Mummy.	Source.	Date when examined.	Species of Fungus present.	Dimensions of Conidia.	
				Range of Size.	Average Size.
Peach	Ontario	April 4	<i>S. americana</i>	$9 \times 7.5-22 \times 16$	16.5×12.2
Plum	"	April 11	<i>S. americana</i>	$11 \times 8.5-24.5 \times 19$	17.2×12.3
Peach	Virginia	April 8	<i>S. americana</i>	$10 \times 8-18.5 \times 11.5$	14.4×9.8
Apricot	California	Feb. 29	<i>S. cinerea</i>	$6 \times 5-15 \times 10$	11.4×7.8
Peach	"	Mar. 1	<i>S. cinerea</i>	$6 \times 4.5-16 \times 10.5$	11.5×7.9
Peach	Kentucky	Mar. 3	<i>S. americana</i>	$8 \times 6.5-18 \times 14$	13.6×9.1

SUMMARY.

The present distribution of the common brown-rot fungi, so far as has been ascertained from the literature on the subject and a study of strains collected by the writer, is as follows:

Sclerotinia fructigena: Europe, Japan, Manchuria.

Sclerotinia cinerea f. *pruni*: Europe, the Pacific coast of North America, Manchuria, and (according to Takahashi) a form of *S. cinerea* occurs on various species of *Prunus* in Japan.

Sclerotinia cinerea f. *mali*: Great Britain and Ireland (and probably the Continent).

Sclerotinia americana: the United States, British North America, Australia, and New Zealand.

The economic significance of this distribution is discussed.

In conclusion the writer desires to express his indebtedness to all those plant pathologists who have given most willing assistance in this investigation by sending him specimens or cultures.

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A New Theory of the Morphology of the Calamarian Cone.

BY

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IN attempting to explain the morphology of the cones of the Calamariae, modern botanists have usually either regarded bracts and sporangiophores as lobes of a dorsiventrally divided sporophyll, or they have held the bracts to be sterilized sporangiophores, or the sporangiophores to be fertile leaves. But another view is possible. It may be that in the primitive Equisetales the cones consisted of successive whorls of sporangiophores (perhaps interrupted at considerable intervals by whorls of ordinary leaves), and that, in the evolution of certain types, whorls of leaves, resembling those borne by the branch of which the axis of the cone formed the prolongation, were produced also on this prolongation. Eventually, the leaves would seem in certain forms (*Calamostachys*, *Palaeostachya*) to have come to be intercalated regularly between the whorls of sporangiophores and to have assumed a protective function towards the developing sporangia. This is the theory that seems to me the most probable, and I propose briefly to discuss it.

SECTION I. THE RELATIVE AGES OF BRATTLESS AND BRACTEATE CONES.

The theory outlined above is supported by the greater antiquity of the bractless cones. *Pothocites*, Paterson,¹ *Pothocitopsis*, Nath. (36, pp. 77-8), and *Asterocalamites scrobiculatus*, Schl.² are characteristic of the very oldest carboniferous rocks, while cones in which whorls of sterile bracts intervene between the whorls of sporangiophores (*Calamostachys*, *Palaeostachya*, *Cingularia typica*, Schpr.) have not, with the exception of the little known

¹ Though some writers, e.g. Scott (47, p. 63), speak of the occasional sterile whorls found at considerable intervals on the axis of the cone as composed of *small* leaves or bracts, these whorls appear to consist of unreduced—or almost unreduced—leaves.

² This form is sometimes regarded as specifically identical with *Pothocites* (29, p. 312; 50, p. 385; 25, p. 35; 27, p. 80); but the much greater size of the cones of the latter and, above all, the dichotomy of its cone-bearing axes seem to preclude this identification. See, however, 38, p. 212, for a bifurcating axis ending in two small cones of *Bornia radiata*, Ren. (= *Asterocalamites scrobiculatus*, Schl.).

Calamostachys occidentalis, Bureau (12), been found below the Upper Carboniferous. The bractless cones did not die out with the end of the Lower Carboniferous; indeed, they alone have survived to our day in the genus *Equisetum*. Leaving out of account some rather doubtful types described by Grand'Eury (16, p. 41; 17, p. 223), we find in the Upper Carboniferous bractless cones belonging to such various members of the Equisetales as *Autophyllites furcatus*, G.E., *Cingularia Cantrilli*, Kidst., Cantrill and Dixon, and *Equisetites Hemingwayi*, Kidst. From rocks transitional from the Palaeozoic to the Mesozoic Age we have *Phyllothea deliquescens*, Goepp, strongly reminiscent of *Pothocites*,¹ while in the Mesozoic and Tertiary beds we find a number of bractless cones of *Equisetites* (18 and 37), a genus of which some Tertiary species are practically indistinguishable from existing species of *Equisetum*.

SECTION II. THE LEAFY STRUCTURE OF THE BRACTS.

The leafy nature of the bracts in the cones of many Calamariae is very striking and was emphasized as long ago as 1909 by Dr. H. H. Thomas for *Calamostachys Binneyana*, Carr. (53, p. 253). It must be remembered that Calamite cones often terminated small branches, the leaves borne by which were also small. It is significant that Dr. H. H. Thomas should state that the small leaves described by him, which were from 1.5 to 2.5 mm. long, occurred chiefly in slides prepared to show cones of *Calamostachys* (54). In the slender *Calamostachys*, with long internodes, borne by *Asterophyllites heimansi*, Jongmans and Gothan, the bracts are described as similar to and disposed in the same way as the leaves (28, p. 67, Pl. X, Figs. 11 and 12). In some forms the bracts were long and leaf-like. Thus, in *Calamostachys Solmsi*, Weiss, they were about 7 mm. long; in *C. Zeilleri*, Ren., they are said to be 10 mm. long and to possess secondary xylem in their bases (40, p. 408); in *Paracalamostachys striata*, Weiss, they seem to have been over 12 mm. long (56, p. 192, Pl. XX, Fig. 5), while in *Palaeostachya superba*, Weiss, they were about 18 mm. long and are frankly termed leaves by Weiss (55, p. 46). Similarly, M. l'Abbé Carpentier, in describing some petrified structures closely resembling the bracts of *Calamostachys* (*Bruckmannia*) *Grand'Euryi*, Ren., goes so far as to write that he is inclined to see in these foliar organs bracts and (*sic*) perhaps leaves of *Annularia* (14, p. 243).

It is, of course, true that in some cases the intercalated leaves have become much modified, probably for protective purposes (e. g. *Calamostachys Grand'Euryi*, Ren., *C. borgiensis*, Ren., *Palaeostachya* (*Huttonia*) *spicata*, Stbg., *P. gracilis*, Ren.), or much reduced in size, as often occur when leafy

¹ See, especially, Schmalhausen's Fig. 17 of Pl. IX (42), accepted by Jongmans (27, p. 657) as of *Phyllothea deliquescens*, though queried by Solms-Laubach (51, p. 181).

structures are associated in a strobilus (e. g. *Calamostachys tuberculata*, Stbg., with bracts but 4–6 mm. long, while the leaves of the cone-bearing branch are 1.5 cm. long). These are, however, clearly derivative types, and in many species the leafy nature of the bracts is very obvious.

SECTION III. THE MORPHOLOGICAL INTERPRETATION OF THE CONES.

The view that the bracts of the Calamariae were original constituents of the cones of the phylum leads to difficulties of interpretation, especially in the case of the bractless types. It has been suggested that the sporangio-phores of such types as *Asterocalamites* and *Equisetum* have arisen by the fusion, during phylogeny, of dorsal and ventral lobes of a sporophyll, the former being regarded as the homologues of the sterile bracts in *Calamostachys* and *Palaeostachya* (24, pp. 184–5). A study of the sporangio-phores of *Equisetum* certainly does not support this view. Double sporangio-phores, i. e. sporangio-phores showing various degrees of concrescence, are not uncommon in the genus (5, p. 674–5; 9, pp. 601–2); but, with the exception of very rare abnormalities, the concrescence seems always to be in a lateral direction, i. e. between members of the same whorl. Even in large cones of *E. maximum*, in which the sporangio-phores were very irregularly arranged and certain whorls appeared to have lost their identity, there is no indication of a dorsiventral duplication of individual sporangio-phores (6, p. 232, Pl. XIII). Moreover, the sporangio-phores of such a type as *Asterocalamites* are so closely similar to those of some species of *Calamostachys* that it is almost inconceivable that in the former they should represent the fused bracts and sporangio-phores of the latter.

A second view, that the sterile bracts of the cones of certain Calamariae have become extinct in *Asterocalamites* and *Equisetum*, was suggested by Dr. Jeffrey as an alternative to the view just discussed (24, pp. 184–5) and was regarded as a possible solution by Lignier, who, however, inclined rather to the view that the sporangio-phore was sometimes a whole organ and sometimes a lobe of an organ (34). It certainly seems very unlikely that the bracts should have vanished completely, leaving no trace externally or in the anatomy; and still more unlikely that these bracts, present in many cones from the Upper Carboniferous, should already completely have disappeared from all the cones of the Lower Carboniferous, except in *Calamostachys occidentalis*, Bureau. Then, there is the difficulty subsequently raised by the discovery of *Cingularia Cantrilli*, Kidst., Cantrill and Dixon. This cone is of the same age (Upper Carboniferous) as the well-known *C. typica*, Schpr., and so closely resembles it that, except for the absence of any sterile whorls between the whorls of sporangio-phores, the cones appear to be indistinguishable (31, p. 1045). In particular, the highly peculiar, strap-shaped sporangio-phores, unknown elsewhere in the Vegetable Kingdom, are

identical. Can we suppose that all trace of the bracts has disappeared within the relatively narrow circle of affinity of these two species of *Cingularia*?

A third view is that the bractless cones, the sporangiophores of successive whorls represent alternately dorsal and ventral lobes of a dorsiventrally compound sporophyll of which both lobes were fertile, as were those of the sporophyll of *Sphenophyllum fertile*, Scott. This view was first tentatively suggested, but later abandoned, by Dr. Scott (45, p. 162; 46, p. 623). I, for a time, held this view, with the added deduction that the fertility of both lobes of the dorsiventrally divided sporophyll was primitive (4, pp. 3-4), a view which enables us to explain the absence of bracts (i. e. of sterile lobes) in the older types and affords an explanation of the different position of the bracts with reference to the sporangiophores, since sterilization could be held to affect the dorsal lobes in *Palaeostachya* and *Calamostachys* (the ventral ones being vertically displaced in the latter, see pp. 309-11) and the ventral lobes in the cone of *Calamites paleaceus*, Stur. (30, p. 110), and *Cingularia typica*, Schpr., both lobes remaining fertile in *C. Cantrilli*. This theory, however, can hardly be applied to *Equisetum*, where the anatomy of the cone indicates that the sporangiophores are whole organs. Finally, it should be emphasized that there is, so far as we know, no Equisetalean cone in which bracts and sporangiophores form part of a dorsiventrally divided sporophyll.

Fourthly, there is Dr. Goebel's view that the sporangiophores are fertile leaves (15, pp. 1104-10). To him the homology of leaves and sporangiophores is so clear that the difficulties of interpretation of the fossil cones cannot prevail against it. It is curious that he, who is so convinced a supporter of the foliar nature of the Equisetaceous sporangiophores (his sporophylls), should interpret such leafy structures as the Calamarian bracts as sterilized sporangiophores. He regards the sporangiophores of *Equisetum* as homologous with those of *Calamites*, and thinks it probable that the Calamitean cones arose from Asterocalamitean (his Archaeocalamitean) forms by the vegetative development of certain whorls of sporangiophores (his sporophylls), leading to the protection of other whorls. The young sporangiophores, profiting by the increased protection afforded them, might have come to originate later and in smaller numbers, between two vegetative whorls, a process for which he finds an analogy in the evolution of certain angiospermous flowers. In such a case it would not, he holds, be surprising if the whorls of leaves alternated with one another and not with the sporangiophores, adding that, on general grounds, it is only a physiological alternation that is to be expected—that is, an alternation of organs making similar demands for constructive material.¹ Of Dr. Goebel's further argument that, by assuming,

¹ The significance of the insertion of bracts and sporangiophores on independent systems is discussed in the next section.

as he does, hyponasty of the sporangiophoric primordia, combined with other changes in the localization of growth, with reduction of the foliar laminae, with chorisis of leaf-primordia or with other changes in their relative positions, it is possible to regard the sporangiophores as modified foliar organs, it can only be said that, with the help of such far-reaching assumptions, it would be easy to discover possible homologies. The argument founded on the existence of abnormal structures, apparently intermediate between leaves and sporangiophores, certainly has some weight (15, Fig. 1074, I and III; Fig. 1076, I and IV; Fig. 1089).

Among the Equisetaceae, malformations, especially fusions of sporangiophores, are not uncommon, but it is, probably, unsafe to attach much value to them as a key to morphology. It would not be the first time that such variations have been overestimated in judging affinity (see Dr. Scott (48, pp. 396-8) for the case of the Psilotaceae). The occurrence of a normally sporangiferous annulus in *E. xylochaetum* (35, p. 383) and, since the publication of the last edition of Dr. Goebel's 'Organography', also in *E. giganteum* (7, p. 254), might be used in support of the view that the sporangiophores are foliar organs, since the annulus is, as Dr. Goebel points out, a reduced foliar whorl (see also 7, pp. 256-8). It would seem, however, that the sporangiferous annulus is not primitive. Its occurrence only in two closely related species¹ would seem to indicate that it has been acquired relatively recently and within the limits of the genus. Again, the absence in *Equisetum giganteum*² of any annular or supra-annular branching of the axial strands, such as we find in the other species whose anatomy is known (except in the small, obviously reduced cone of *E. variegatum* (8)), suggests that the vascularization of the annulus has placed a considerable strain on the xylem-producing capacity of the axis in this region, and that this has led to relatively poor development of vascular tissue, exemplified, as such reduction frequently is, by loss of anastomosis. Lastly, and this is the weightiest argument against the primitiveness of the sporangiferous annulus, the geological record shows that, even if leaves and sporangiophores are ultimately homologous, the distinction between them is much older than the evolution of the peculiar Equisetaceous leaf-sheaf. Consequently, even if the annulus represents a primitively sporangiferous whorl, its similarity to a reduced leaf-sheath is due to homoplasmy and cannot be used to support the foliar nature of the sporangiophore (7). Thus, though the leaves of the existing Equiseta are highly modified structures, the fact remains that, as far back as we can trace the Equisetales, their leaves and sporangiophores are strikingly different. Further, while malformed whorls of leaves and sporangiophores are not uncommon in *Equisetum*, there is no known Calamarian

¹ In Milde's classification (35), *E. giganteum* and *E. xylochaetum* are placed next but one to one another in the subgenus (his genus) *Hippochaete*.

² The anatomy of the cone of *E. xylochaetum* is unknown.

cone in which the bracts show any sign of having arisen from the sporangio-phores; and in the whole phylum of the Equisetales there is no organ which, when normally developed, is really intermediate in structure between leaves and sporangio-phores, though the sporangiferous annulus shows a physiological approach to a fertile whorl.

Finally, the sporangio-phore has been held by Dr. Scott and others (46, p. 623; 34, p. 131; 5, p. 697) to be sometimes a leaf, sometimes a lobe of a leaf. More recently, Dr. Scott has restated this position in somewhat wider terms, for, after remarking that the analogy with *Sphenophyllum* may well have been pushed too far, he writes that it is possible that in the Sphenopsida 'the spore-bearing organ may be sometimes a lobe of a leaf, sometimes an entire leaf, or not of foliar nature at all' (48, p. 407). In agreement with Drs. Kidston and Lang (32, p. 850), he suggests that when the sporangio-phore is not of foliar nature it may be directly derived from a fertile thallus branch, such as those found in the Psilophytales. *Asterocalamites* is quoted as an example of a plant the sporangio-phores of which have perhaps not passed through a definitely foliar stage (48, pp. 408-9). It might be argued that the genus *Cingularia* supplies a proof of the validity of Dr. Scott's suggestion that the sporangio-phore may be either a leaf (*C. Cantrilli*) or a lobe of a leaf (*C. typica*). He has, however, himself pointed out that it is probable that in certain lines of descent the differentiation of the sporangio-phore took place very early and that the organ is thus of considerable morphological importance (46, p. 624). This is emphatically true of the Equisetales, in which typical sporangio-phores occur in the oldest known cones of the group (*Asterocalamites*, *Pothocites*) and were widely distributed in later ages in cones of such different types as those of *Palaeostachya*, *Calamostachys*, *Phyllothea*, and *Equisetites*. It seems, therefore, impossible to believe that the peculiar and presumably specialized type of sporangio-phore found only in *Cingularia*, a genus of no particular antiquity among the Equisetales, is not only a primitive, morphologically indeterminate organ, but also that the bracts were evolved as fresh, sterile lobes or completely disappeared within the relatively narrow limits of affinity of the geologically contemporary and otherwise closely similar *C. Cantrilli* and *C. typica*.

To accept the view that the sporangio-phores of the obviously natural group of the Equisetales may be leaves, leaf-lobes, or non-foliar will admittedly enable us to describe in apparently morphological terms the cones of the phylum or of almost any conceivable combination of sporangio-phores and other organs. But the description would have little morphological or phylogenetic value; and to accept a definition depriving the sporangio-phores of the Equisetales of any morphological status would be to admit defeat in the attack on the evolutionary problem presented by the cones of the group.

SECTION IV. THE RELATIONS OF NUMBER AND POSITION BETWEEN THE MEMBERS OF FERTILE AND STERILE WHORLS.

Professor Bower has frequently emphasized the fact that there is no definite relation, either of number or of position, between bracts and sporangiophores (3, pp. 153, 383, 426). The sporangiophores may stand in the axils of, or a little above, the bracts (*Palaeostachya*); or midway between two sterile whorls (*Calamostachys*); or immediately below the bracts (*Cingularia typica*, Schpr.), cone of *Calamites paleaceus*, Stur. (30, p. 110), *Volkmannia pseudo-sessilis*, G.E., and the cone attributed, probably erroneously, by Schenk to *Annularia sphenophylloides*, Zenker (his *A. brevifolia* (41, 16, p. 43; 25, p. 296)). Further, the bracts may be equal or approximately equal in number to the sporangiophores (*Palaeostachya vera*, Sew.); or there may be typically three bracts to every two sporangiophores (*Calamostachys magnae-crucis*, Browne); or, and this seems the commonest combination, the bracts may be twice or approximately twice as numerous as the sporangiophores (*C. Binneyana*, Carr., *C. Ludwigi*, Carr., *C. tuberculata*, Stbg., *C. Grand'Euryi*, Ren., and probably *C. calathifera*, Weiss (see 10, p. 354), *Palaeostachya Ettingshauseni*, Kidst., *P. gracilis*, Ren., and a *Palaeostachya* described by Renault as *Volkmanni equisetiformis* and erroneously attributed by him to *Asterophyllites equisetiformis*, Schl.). But certainly in some and probably in most of these species the number of members in a whorl varied somewhat, and there is evidence that this variation in number was not always parallel in the two kinds of organ, even in neighbouring whorls. This was so in *C. Grand'Euryi* and *C. magnae-crucis* (10) and in *C. Binneyana* (21, p. 11; 22, p. 229). In regard to the last species Dr. Hickling, although he held that bracts and sporangiophores represented lobes of a dorsiventrally divided sporophyll, particularly pointed out that no definite numerical relation could now exist between them, as the commonest number of bracts was thirteen. And in an earlier paper on *Palaeostachya vera* he admitted that bracts and sporangiophores, having become vertically separated on the axis, might be multiplied independently and in some cases irregularly (20, p. 383). A further important indication that the bracts were not original constituents of the cone is afforded by the fact that they and the sporangiophores are inserted on the axis on independent systems. In the majority of Calamarian cones the bracts of successive whorls alternated with one another, while the sporangiophores were superposed to one another. This was so in *Palaeostachya gracilis*, Ren.,¹ in *P. Ettingshauseni*, Kidst., in *C. magnae-crucis*, Browne, in *C. cala-*

¹ This alternation is clearly shown in Figs. 3 and 4 of Pl. XXVIII of Renault's Atlas (39). I have verified the alternation on the original specimens. If Fig. 5 of the same plate is of *P. gracilis* it is incorrectly drawn, both as regards the superposition of the bracts and the position of the sporangiophores midway between the sterile whorls.

thifera, Weiss (10, p. 354), in *C. tuberculata*, Stbg. (11, p. 306-7), in *C. Ludwigii*, Carr., in *C. Binneyana*, Carr., and probably in many others. Dr. Hirmer has recently shown that in the last two species the bracts, where they are accurately twice as numerous as the sporangiophores, lie in *alternate* whorls in pairs opposite to the intervals between the sporangiophores, while in the intervening whorls they lie opposite to both flanks of the peltate heads of the sporangiophores, as seen in a transverse section of the axis (22). This relation only holds good in those cases, possibly a minority, in which the bracts are exactly twice as numerous as the sporangiophores. This is all that Dr. Hirmer claims in his definition of the position of the bracts, although in one or two places he attempts, rather unconvincingly, to apply his scheme to whorls in which this proportion does not hold good, e. g. to the whorl shown in his Text-figure 7, p. 232 (22). Dr. Hirmer's work seems to support the view, not held by him, that the bracts are phylogenetically intrusions into the cone. For, if we attempt to imagine what would occur if the axis of a cone of the *Calamostachys Binneyana* type, but without nodal structure and sterile whorls, were to produce, between the fertile whorls, whorls of protective leaves twice as numerous as the sporangiophores, we shall realize that the most natural position for these new appendages, requiring vascular connexions with the axis, would be for them to be equidistant and to lie on either side of lines passing radially through the sporangiophores, of which a pair stood opposite to each bundle. That is, the intruding organs would occupy positions identical with those of the bracts in Dr. Hirmer's first position. Agreeably to the rule of alternate verticillation, prevalent in most Calamariae, the leaves of the next whorl would alternate with these, provided that their number remained constant, and would, therefore, occupy positions identical with those of the bracts in Dr. Hirmer's second form of disposition.

Though alternation of the bracts seems to be the rule, it would appear probable that in some forms leaves were produced regularly between the sterile whorls by the axis at a period before the primitive superposed phyllotaxy of the Equisetales had been lost, and that the relatively conservative reproductive axis retained the superposed phyllotaxy after it had been lost by the vegetative axes. For, in a few forms, the bracts of successive whorls seem to have been superposed to one another, while the sporangiophores were also superposed to one another, though not to the bracts. This appears to have been so in *Calamostachys Grand'Euryi*, Ren., and *C. (Arthropityostachys) borgiensis*, Ren.¹ In two species a superposition of sporangiophores to bracts has been suggested, in a *Palaeostachya* figured by Renault as 'portion d'épi d'*Astérophyllyte*' (39, Atlas, 1893,

¹ Dr. Hirmer (22, p. 246) relies upon Renault's figures to prove alternation of the bracts in *C. Grand'Euryi*. Renault's Fig. 3 of Pl. LXII (39), however, hardly supports this view, while the originals show superposition of bracts (10, p. 339).

Pl. XXX, Fig. 1), but not mentioned in his text, and in *P. vera*, Sew. The sporangiophores of the former type are drawn as though superposed to the superposed and spurred bracts; but in the Renault collection in Paris I was able to see that the original section, of which Renault's figure is a restoration, does not traverse the stalks of the sporangiophores. In one place the proximal end of a stalk appears to be visible, but on careful focusing it can be seen that this projection represents a torn portion of the axis. Of *Palaeostachya vera* Dr. Hickling merely says that its sporangiophores were approximately equal in number to its bracts and probably superposed to them, though he could not determine this with any certainty (20, pp. 372 and 375). Consequently, with this possible but doubtful exception, in all Calamarian cones in which the relative positions of bracts and sporangiophores are accurately known, these organs were inserted on the axis on independent systems and frequently varied in number independently. Were bracts and sporangiophores lobes of a dorsiventrally divided sporophyll we should expect that where the bracts were double the number of the sporangiophores the latter would be inserted regularly opposite to the interval between every two bracts. Were the sporangiophores whole foliar organs one would expect that where they were half as numerous as the bracts they would alternate with pairs of these (3, p. 383).

It is probably significant that in all the Equisetales, except *Phyllothea deliquescens*, Goepp, that survived the close of the Palaeozoic Age without developing bracts between the whorls of sporangiophores, the latter seem to have alternated in successive whorls. This alternation, combined with the imbrication of the heads of the peltate sporangiophores, leads to the development of a very perfect protective armour, persisting until the spores are mature and the axis between the whorls elongates. This can be well seen in young cones of most species of *Equisetum*, though in those with large cones, especially in *E. maximum*, the alternation may be obscured or lost owing to the irregular disposition of the sporangiophores (15, p. 1104). On the other hand, we know of no certain case of alternation of sporangiophores where the developing sporangia were protected by bracts.

SECTION V. THE ANATOMY OF THE CONES.

Unfortunately no fossil bractless cone is known in structural material.¹

It is, perhaps, largely the position of the sporangiophores in *Palaeostachys* that has led a number of botanists, influenced by the analogy with the Sphenophyllales, to regard the sporangiophores of the Calamariae as ventral lobes—vertically displaced in *Calamostachys*—of sporophylls of

¹ Hickling's statement that petrified cones of the *Bornia* type are known (20, p. 380) and Jongmans's reference to petrified cones of *Bornia esnostensis* (27, p. 84, under *Asterocalamites scrobiculatus*) seem to be erroneous. As pointed out by Dr. Scott (47, p. 63), cones of this type are unknown petrified.

which the bracts represent the dorsal lobes. The force of this analogy has been seriously weakened since Dr. Hickling showed that in *P. vera*, Sew., the traces of the sporangiophores originate just above those of the bracts of the whorl below, ascend half-way up the internode in contact with the axial bundles, and are then sharply reflexed, descending through the cortex until they enter the more or less axillary sporangiophores (20, pp. 375-6). This course of the traces of the sporangiophores of *P. vera* caused Dr. Hickling to regard *Palaeostachya* as a derivative of *Calamostachys*, a view which has been widely accepted (46, p. 622; 48, p. 406; 52, pp. 82-3; 4, pp. 16 and 116).¹ It now seems to me that on the evidence supplied by *P. vera* there is no need to regard *Palaeostachya* as a derivative from *Calamostachys*. The intruding bracts may have been originally produced in different positions, relatively to the sporangiophores. Dr. Hickling has pointed out that there is no sign that the extensions of the sclerenchymatous nodal disc filling the angle between the ascending and descending limbs of the trace of *P. vera* were upgrowths that had carried up the traces. But the upward direction of the trace of the sporangiophore in the inner part of its course may well have been a response necessary to surmount these upward extensions. There is no evidence that a similar reflexion of the traces occurs in other species of *Palaeostachya*. Though Dr. Hickling regarded the axillary position of the sporangiophores in *Palaeostachya* as secondary, he held that the evidence from *Calamostachys* itself justified the view that the sporangiophores were in both genera ventral lobes of sporophylls, of which the bracts were dorsal lobes, a view accepted at one time by Dr. Scott (46, p. 622, and 48, p. 406) and by myself (4, pp. 16 and 116). Dr. Hickling admits that in *Calamostachys Binneyana* the traces of the sporangiophores only separate from the axial bundles at or near the level of these organs, but holds that there is no good evidence that they really arise from the stele at this level. He claims, firstly, that the trace seems not to be connected with the axial protoxylem at this level, and that in one section a trace could be followed uninterruptedly down to the subjacent node—that is, I estimate, for a distance of from 0.3 to 0.5 mm.; secondly, that, in other cases, traces may be seen projecting from the bundles below the level of the sporangiophores; and, thirdly, that, in yet other cases, he has observed within the axial bundles indications of small independent protoxylem canals (21). All these phenomena, however, merely suggest an early preparation for the departure of the trace and can be observed also in the cones of *Equisetum*. Their occurrence in cones devoid of bracts shows that they afford no presumption that the sporangiophores are displaced ventral lobes of a sporophyll. Thus, in the lower region of a mature cone of *E. maximum* the protoxylem may separate as much as 2 mm., and frequently separates as much as 0.5 to 0.75 mm. below the level of its departure from the bundle (9, pp. 597-8).

¹ See also 49, pp. 41-2, for Dr. Lashevsky's endorsement of this view.

Again, in the lower part of mature cones of several species of *Equisetum* the protoxylem of the trace is often represented in its course within the bundle by a small canal. Lastly, though the traces usually become free as soon as their xylem has reached the periphery of the bundle, I have observed that in *E. maximum* the traces may project for a considerable height as ridges on the outside of the bundles before they depart.

Calamostachys Binneyana is a small, simply constructed cone, and probably one of the more primitive of the bracteate types. Its anatomy agrees very well with the view that the bracts are later intrusions into the cone. Though in it the bracts are much more numerous than the sporangiophores, though secondary zylem and nodal structure are confined to the neighbourhood of their insertion, the course of the vertically continuous bundles and their number and position are correlated to the position of the superposed sporangiophores and quite unaffected by the varying number and the position of the alternating bracts. The bundles (usually double) give off traces to the two sporangiophores opposite to which they stand.¹ In contrast to this systematic method of emission of sporangiophore traces, the traces of the bracts depart very irregularly—one might be tempted to call their mode of departure haphazard or opportunist. As there were from twelve to fifteen bracts in a whorl, thirteen being the commonest number, it is clear that, owing to the combined effect of frequent odd numbers and of alternation of the bracts in successive whorls, the bundles must, more often than not, not have stood opposite to pairs of bracts. Some bundles gave off three, others four traces, and these sometimes underwent torsion in the cortex, doubtless in order to reach the bracts (21, p. 10, Text-figure 3). Dr. Hirmer writes that on purely theoretical grounds it can be said with certainty that in *C. Binneyana* there must have been two types of bract-trace course occurring alternately in successive whorls and corresponding respectively to the two types of position of the bracts with reference to the sporangiophores recognized by him (see p. 308). He supposes that when the bracts lie opposite to each of the flanks of the heads of the sporangiophores, as seen in a transverse section of the axis, each double bundle lying opposite to a pair of sporangiophores gave off two traces, one from each half, and that these subsequently forked, each resulting bundle entering a bract. He refers to a figure of Dr. Hickling's as supporting this, though the figure in question (21, Text-fig. 3, p. 10) shows clearly both the torsion of the traces and the fact that in this specimen only one bundle gave off four traces. Dr. Hirmer admits that the method of supplying traces to the bracts is not definitely ascertained for those whorls in which the bracts are alternately opposite to the sporangiophores and to the intervals between the latter. He suggests, however, that it is probable that

¹ Occasionally the two halves may, apparently, separate for a while to form distinct bundles (21).

here, too, every bundle gave off two strands ; that each of these then divided into three branches, the middle strand in each group of three entering a bract opposite to a sporangiophore, while each of the others fused with a similar neighbouring strand belonging to the other member of the original pair, the strands resulting from this fusion entering the bracts opposite to the intervals between the sporangiophores (22, pp. 237-8 ; Text-figs. 3 and 4, p. 230). Such copious branching and fusion, admitted by Dr. Hirmer to be purely hypothetical, would seem to be very improbable, even were there no evidence against it. Nor would it explain the mode of vascular supply of the bracts in those cases, said to be the commonest, where there are thirteen bracts in a whorl. Moreover, we know from Dr. Hickling's work that the mechanical difficulty of supplying traces to bracts varying in number and alternating in position from a relatively constant number of bundles¹ was met by some bundles giving off more traces than others and by torsion of some of the traces in the cortex. A similar simple method of adjustment of the traces (this time those of the sporangiophores) is found in some species of *Equisetum*, where the axial bundles anastomose relatively poorly and are narrow, so that the traces are frequently not given off on the radii of the sporangiophores they supply (6).

Calamostachys Casheana, Will., *C. Ludwigii*, Carr., and, from the very little we know of it, *C. Oldhamia*, Hick et Lomax (19), seem to have been constructed on the same general lines as *C. Binneyana*, while *C. tuberculata*, Stbg., though built on a much larger scale, seems to have been essentially similar anatomically, except that the bundles were equal in number to the sporangiophores (11). Two Calamarian cones from the French Coal Measures, first described by Renault, *C. Zeilleri*, Ren., and *C. magnae-crucis*, Browne, were alike in many ways.² We do not know whether the bracts and sporangiophores of *C. Zeilleri* alternated with or were superposed to one another,³ nor what was the course of its vascular bundles ; but in *C. magnae-crucis* the bracts of successive whorls alternated with one another, while the sporangiophores were superposed, except where a change in their number caused a disturbance of this arrangement. There is some evidence (10, pp. 316-17) that in *C. magnae-crucis* the bundles alternated at the nodes. Such alternation might be used as an argument to show that the fundamental structure of the cone was correlated rather to the alternately disposed bracts than to the superposed sporangiophores. Alternation of the bundles of the cones is, however, a rare character in Calamarian cones, and must be regarded as a character acquired relatively recently, possibly under

¹ The bundles seem to have varied in number in different parts of the same cone (21).

² Dr. Hirmer (22, pp. 249-50) assumes that they were specifically identical, but, for the present, the evidence hardly justifies such a view.

³ The longitudinal sections assigned to *C. Zeilleri* by Renault have been shown probably to belong to *magnae-crucis* (10).

the influence of the alternating bracts, rather than as a primitive feature of the cones. Further, the fact that in *C. Zeilleri* and *C. magnae-crucis* the traces of the sporangiophores were given off at, or just above, the level of insertion of the sterile bracts below them, does, to a certain extent, support the view that the sporangiophores are vertically displaced ventral lobes of sporophylls of which the bracts represent dorsal lobes. On the other hand, the number of bundles in the axis of both species is correlated to the number of sporangiophores, being apparently equal to that of the latter in *C. Zeilleri* (39, 40) and half as numerous in *C. magnae-crucis*, while in the latter species, at least, the bracts varied somewhat in number, although there were usually about three bracts to every two sporangiophores (10). A steep upward course of the trace of the sporangiophores is found also in the upper part of the cone of *E. maximum*, where the sporangiophores can hardly be held to be displaced ventral lobes of non-existent bracts! A re-examination of cones of *E. maximum*, previously described (6), showed that in the upper part the traces might depart from the axial bundles as far down as the level of the whorl below that which they supply with traces, or only be given off just below the sporangiophores that they enter. For three whorls of this region, belonging to two cones, the average height traversed by the traces was 0.5 mm. Bearing in mind the small amount of elongation of the axis in this region, we see that the traces arise, on average, about midway between two whorls of sporangiophores. If a bractless cone with such a steeply upward course of the traces of the sporangiophores were to produce protective leaves, or bracts, in the position characteristic of *Calamostachys*, the traces of the two kinds of organ would naturally originate at very nearly the same level. This may well have occurred in the ancestors of *C. Zeilleri* and *C. magnae-crucis*.

Lignier, who regarded the sporangiophores as displaced lobes of a sporophyll, based his interpretation of the morphology of Calamarian cones largely on the anatomy of *C. Zeilleri*, known only from Renault's description. Lignier's account, however, of the course of the traces of the sporangiophores contains two serious inaccuracies, which vitiate his conclusions, even for the species to which they are held to be most clearly applicable. Renault says that the fourteen sporangiophores are supplied with traces which depart from the fourteen bundles, one from each, just above the departure of the bract-traces, of which each bundle gives off two (39, p. 30). Lignier, however, firstly, makes each of the fourteen axial bundles give off *two* sporangiophore-trace-bundles and then introduces a fusion of these in pairs, the fusing members being described as arising from neighbouring bundles (34, pp. 126-7 and Text-fig. 7 a). He is thus enabled to argue that the group of four sporangiophore-trace-bundles (fusing in pairs) given off by two neighbouring bundles, with the group of four bract-traces arising just below from the same two bundles, belong to a dorsiventrally and laterally divided highly com-

pound sporophyll, of which the vertically displaced ventral lobes have fused in pairs to form sporangiophores. This conception is, however, in contradiction with Renault's account, and Renault alone, so far as we know, has studied the original sections.

Thus, though the course of the traces of the sporangiophores of *C. Zeilleri* and *C. magnae-crucis* is compatible with the view that the sporangiophores are ventral lobes, it can also be interpreted in another way; while the independent variations in number of bracts and sporangiophores make it very improbable that they are lobes of the same sporophylls.

C. Grand'Euryi, Ren., is a highly complex cone, obviously far from primitive. The bracts are highly modified structures and the axis contains two kinds of bundles, both vertically continuous: larger deeper-seated ones, approximately equidistant, separated occasionally by one, but usually by two smaller, more peripheral bundles. These are usually equal in number to, and run opposite to, the sporangiophores, to which they give off traces at or just above the level of insertion of these organs. Only exceptionally, when the sporangiophores are more numerous than the bundles, do the main bundles take over the task of supplying the supernumerary sporangiophores with traces. At the node the bigger bundles give off two, the smaller usually one but occasionally two, bract-trace-bundles.

The most likely interpretation of the smaller, more peripheral strands—as yet unknown in other Calamariae—seems to be that which regards them as former traces, that, like those of *C. Zeilleri* and *C. magnae-crucis*, pursued an obliquely upward course through the lower half of the internode, but which, unlike these, have come to persist not only through the upper part of the internode, but even through the node, and have thus become true axial strands, giving off traces to bracts and sporangiophores. This would explain their peripheral position, their opposition to the sporangiophores, and why the main bundles only exceptionally give off traces to the latter. If this hypothesis is correct, each of the vertically continuous bundles of the axis of the primitive, bractless progenitor of *C. Grand'Euryi* ran opposite to, and gave off traces to, a pair of sporangiophores, these traces probably becoming free at a level midway between the sporangiophores that they enter and those of the whorl below. If the axis of such a cone were to develop whorls of protective leaves, twice as numerous as the sporangiophores, and situated as in *Calamostachys*, and if the sporangiophore-traces were to persist through the upper part of the internode and through the node, merely sending off branches to the sporangiophores and bracts, we should get a cone essentially of the same type as *C. Grand'Euryi* (10, p. 350).

Our knowledge of the anatomy of other Calamarian cones is still too incomplete for them to be considered here.

SECTION VI. SUMMARY AND GENERAL DISCUSSION.

In the previous pages it has been suggested that the vexed question of the morphology of the Calamarian cones may probably be solved by regarding the fructifications as axes bearing whorls of fertile appendages (sporangioophores), between which whorls of protective leaves, occupying various positions relatively to the fertile whorls, were in some types produced during phylogeny. It will, doubtless, occur to many that the generally admitted affinity between Equisetales and Sphenophyllales may be used as an argument against such an interpretation. For do not the sporangioophores of the Sphenophyllales appear to be leaf-borne? And are not these organs often closely similar in both phyla? The sporangioophores of a typical *Calamostachys* and that ancient Sphenophyll, *Cheirostrobis*, are, as Dr. Scott has pointed out, almost identical, and it is difficult to doubt their homology (48, p. 405).

Dr. Thomas, in 1909, pointed out that while the available evidence was inconclusive as to whether the sporangioophore of the Equisetales was a fertile lobe of a sporophyll, a foliar or an axial structure, or an organ *sui generis*, the theory adopted as to its nature ought to rest mainly on data supplied by members of that phylum. 'Evidence', he wrote in the concluding sentence of his paper, 'supplied by other groups may be useful to confirm the conclusions reached, but it is scarcely safe, at present, to argue directly from the species of one group to those of another' (53). The morphology of the cone of the Equisetales has been approached in the spirit of the above quotation. But, though the affinities of the phylum lie outside the scope of the present essay, yet the bearing of the undoubted affinity of Sphenophyllales and Equisetales cannot be altogether neglected, and I propose very briefly to summarize the views as to this affinity to which the conclusions already exposed have led me.

A relationship between the two phyla seems clear and has been widely accepted of recent years (24, p. 184; 34, p. 132; 20, p. 308; 3, p. 713; 46, pp. 619-21; 48, pp. 404-5; 4, p. 113; 23, p. 114; 2, p. 89; 1, p. 74; 33, p. 141). There has, perhaps, been a tendency to overestimate its closeness. Dr. Campbell, however (13, pp. 587-8), lays stress on the anatomical differences between the two groups, and Professor Seward has suggested that, while there is evidence that *Asterocalamites* (his *Archaeocalamites*) and *Sphenophyllum* were probably descended from a common ancestral stock, there is no close affinity between the two plants (50, p. 388).

It is certainly puzzling to find in the Sphenophyllales some sporangioophores, such as those of *Cheirostrobis*, hardly distinguishable from the corresponding organs of *Calamostachys*, while others, such as those of *Sphenophyllum Dawsoni*, Will., are essentially similar to the monosporangiate sporangioophores of such an undoubted member of the Equisetales as *Cala-*

mites paleaceus, Stur. (30, p. 110). Drs. Kidston and Lang have pointed out that the sporangiophores of the Equisetales and Sphenophyllales may represent the last persisting remains of the thalloid branch-systems found in some of the oldest known land-plants, the Mid-Devonian Psilophytales (32, p. 850). Such a view would not, of course, involve the assumption that the Articulatae are descended from the Psilophytales, or even from plants closely allied to them. It merely postulates an ultimate homology between the sporangiophores of the former and sporangiferous branches of the latter. The monosporangiate sporangiophores of *Calamites paleaceus* and of *Sphenophyllum Dawsoni*, as well as the bisporangiate ones of *Bowmanites Römeri*, Solms, certainly seem to be closer to the thalloid sporangiferous branches of such Psilophytales as *Rhynia* and *Hornea* than do the peltate quadrisporangiate types. There are in the Mid-Devonian—that is, a horizon older than any in which undoubted members of the Equisetales or Sphenophyllales have yet been recognized—two forms, *Calamophyton primaevum* and *Hyenia elegans*, recently described by Drs. Kräusel and Weyland and placed by them in the new group of the Proto-Articulatae. In these the sporangiophores seem to be to a certain extent intermediate between the peltate quadrisporangiate and the mono- or bisporangiate types. In *Calamophyton primaevum* the sporangiophores fork distally and each branch apparently bears but a single inwardly directed sporangium. One of Drs. Kräusel and Weyland's figures shows the space between the very short distal branches filled in by a flattening of the stalk of the sporangiophore (33, Text-fig. 27, p. 139), so that the latter recalls in form the sporangiophores of *Pothocites* or *Calamostachys calathifera*, which are not peltate, though, unlike those of *Calamophyton*, they are quadrisporangiate. Drs. Kräusel and Weyland themselves emphasize the similarity of the sporangiophores of *Calamophyton* to those of *Calamostachys*. In *Hyenia elegans*, Kräusel, which they regard as the type closest to *Calamophyton*, each branch of the sporangiophore bears distally 2–3 sporangia (33, pp. 135 and 138). The concrescence of the two short distal branches would give us sporangiophores of the type of *Pothocites*, or even of *Equisetum*. If the peltate, quadrisporangiate sporangiophores originated after this fashion, then the similarity between the sporangiophores of *Calamostachys* and that ancient Sphenophyll, *Cheirostrobus*, is largely due to parallel development. That such parallel development should have led to close similarity is hardly surprising, for the organs affected were presumably homologous and of a fundamentally similar type. On the whole, then, the balance of evidence seems to be in favour of the primitiveness of the monosporangiate or bisporangiate types of sporangiophore, at any rate within the phylum of the Equisetales.¹

Passing from the question of the morphology of the sporangiophore to

¹ The strap-shaped sporangiophores of *Cingularia* may be conjectured to have arisen by the flattening of sporangiophores not unlike those of *Hyenia elegans*.

that of the cone, the evidence, considered from a comparative point of view, seems to show that Sphenophyllales and Equisetales began to diverge from one another before the sporangiferous thalloid branches had been relegated to definite and characteristic positions in the branch-systems.

In the Sphenophyllales all the previously thalloid branches of certain branch-systems underwent webbing or cladodification. Such branch-systems came to serve as photosynthetic organs and, becoming appendicular, formed large leaves. In other branch-systems some of the branches seem to have undergone cladodification, while others, belonging to the same system, retained a more or less thalloid form and a sporangiferous function, though doubtless they, too, underwent some modification. On becoming appendicular with reference to a relatively main axis, branch-systems of this type constituted compound sporophylls. In *Sphenophyllum fertile*, Scott, in which the sporophyll is composed entirely of sporangiophores, all the branches of certain systems that have become appendicular have remained more or less thalloid and sporangiferous, though, as might be expected in the absence of definite sterile laminæ, the sporangiophores show a small laminar development. It has been suggested that the fertility of the dorsal lobes of the sporophyll—sterile in all other known Sphenophyllales—is secondary, chiefly because *S. fertile* is of no special antiquity and because the dorsal position of the sterile lobes is best explained by their protective function, a function unnecessary in this species, where the distribution of the lobes and the form of the laminæ ensure the protection of the developing sporangia (44). While it may well be that the regular differentiation of all the lobes of the sporophyll as sporangiophores only became the rule *pari passu* with the formation of a definite cone and the consequent specialization of the sporophylls, it is difficult to believe that such typical sporangiophores as those of *S. fertile* were descended from typically leafy or bract-like parts.

Although most Sphenophyllales were strobiloid with specialized sporophylls, some were non-strobiloid and had very leafy sporophylls (e.g. *Sphenophyllum characforme* (26), *S. majus*, Bronn., *S. tenuissimum*, Kidst.), and these may well have been primitive.

In the Equisetales the verticillation of the thalloid lobes seems to have been carried farther than in the Sphenophyllales, or at least to have extended to branches of a higher order, since the sporangiophores, presumably homologous in the two groups, have themselves become verticillate instead of forming lobes of verticillate appendages.¹ This seems to be true, too, of Drs. Kräusel and Weyland's Protoarticulatae, for both in *Hyenia elegans* and in *Calamophyton primævum* the sporangiophores are aggregated in whorls on portions of the axis devoid of sterile appendages and apparently

¹ There is some evidence that in *Sphenophyllum emarginatum*, Bgnt., the sporangiophores, though closely approximated to the leaves, were inserted on the axis (48, p. 406).

form lax, bractless cones. Drs. Kräusel and Weyland's suggestion that *Calamophyton* and *Hyenia* are obviously nearer to one another than to any other known plants certainly seems justified. But their further contention that the discovery of the fertile parts of *Hyenia* entirely confirms Nathorst's view, founded on impressions of vegetative parts only of *Hyenia sphenophylloides* from Norway, that this genus was probably related to *Asterocalamites* but more closely to *Sphenophyllum*, is open to objection. The position of the sporangiophores in whorls on the axis and not on the leaves suggests a closer, though not necessarily a close, relation to the Equisetales than to the Sphenophyllales. Of the affinities of *Calamophyton* to the Equisetales Drs. Kräusel and Weyland write: 'As regards the last phylum ("Reihe"), the Equisetales, which includes chiefly the Equisetaceae and Calamariaceae, the sporangiophores of certain species of *Calamostachys* are certainly very like those of our fossil, but in this phylum ("Reihe") fertile and sterile whorls alternate and the structure of the stem is quite different' (33, p. 140). It has been shown in the previous pages that the bractless cones of the oldest undoubted Equisetales were probably more primitive than the cones with alternating fertile and sterile whorls. Thus, though it is certainly better to keep *Calamophyton* and *Hyenia* in a separate phylum, the Protoarticulatae, their affinity was probably rather with the oldest Equisetales, such as *Pothocites* and *Asterocalamites*, than with the Sphenophyllales. The dichotomy of the cone-bearing axis of *Pothocites* is strongly reminiscent of the forking of the axis of *Calamophyton* and of the upper part of *Hyenia*. The dichotomy of *Pothocites* seems to be a vestigial character and one of considerable importance in view of the prevalence of verticillate branching throughout the Equisetales.¹ Again, the narrow forked leaves of Protoarticulatae are found both in the Equisetales and the Sphenophyllales, nor is there any certain indication, in the little we know of the anatomy of *Calamophyton* (that of *Hyenia* is unknown), of a closer affinity with *Sphenophyllum* than with the Equisetales. The xylem seems, it is true, to have been triangular, as in *Sphenophyllum*, but the stele seems to have been medullated, as in the Equisetales.

Whatever view we take of the affinity of these remarkable newly described Protoarticulatae, their discovery greatly strengthens the hypothesis of the relative primitiveness of the bractless cone in the Equisetales. For in these forms from the Middle Devonian we have plants not unlike the Equisetales in general build, and possessing bractless cones composed of whorls of sporangiophores markedly reminiscent of those of the Equisetales.

¹ A supposed abnormal forking of the cone-bearing axis has been recorded in *Calamostachys sarana*, Weiss (48), and abnormal dichotomies are known in *Equisetum*.

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The Cytology of *Pyronema domesticum*, (Sow.) Sacc.

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With Plate XVI.

THE organism which forms the subject of this inquiry is closely allied to *P. confluens*. My material, for which I am indebted to Mr. B. Barnes, was found growing on damp wall-paper in the spring of 1923. It proved readily amenable to artificial culture conditions, and cytological work was begun early in 1924. The culture methods were those used by Claussen¹ (3) for his investigation of *Pyronema confluens* (loc. cit., p. 6).

Monosporous and polysporous cultures were made, but there were no observable differences between them. Supplies of material in all stages of development were available in profusion, but it may be pointed out that once a culture has produced apothecia it is useless to look for early stages. On the third day after infection in favourable conditions there will be abundance of sexual organs, but on the fourth day it will be almost impossible to find material not showing ascogenous hyphae.

METHODS.

The young stages, including the formation of ascogenous hyphae, were fixed in Merkel's fluid. For the nuclear divisions in the ascus Flemming's strong fluid diluted with an equal volume of water was used. The material was dehydrated by the very close alcohol series now advocated, and taken into paraffin wax by a similarly close xylol-alcohol series. Sections were cut at thicknesses varying from 3μ to 15μ , but 5μ proved most useful for the younger stages and 8μ for the ascus divisions. Flemming's triple stain (safranin, gentian violet, orange) was used for the Merkel-fixed material, and Breinl's triple or Heidenhain's iron-haematoxylin for the ascus divisions.

¹ A curious error in a chemical formula in Claussen's paper has led to some confusion and has been copied. The iron salt used has the formula $\text{Fe}_3(\text{PO}_4)_2$ and not $\text{Fe}_2(\text{PO}_4)_3$.

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Breinl's triple (10), though a little 'chancey', gives results of magnificent transparency and sharpness. Other fixatives (Bouin's, Carnoy's, various formalin-containing mixtures) and stains (iodine green and fuchsin S., various haematoxylin, cyanin, and erythrosin, &c.) were used for purposes of comparison, but the results to be detailed were not obtained from such material. Orange G in clove oil used lightly, or no counter-stain at all, proved most useful with material stained in Heidenhain's haematoxylin.

SPORES.

The spores of the fungus are smooth, fairly thin walled, and hyaline. It is therefore possible to make preparations of germinating spores showing seven chromosomes in their nuclei. This is the haploid number.

FERTILIZATION STAGES.

The sexual organs present much the same superficial appearance as those described for *Pyronema confluent* by de Bary (2), Harper (8), and Claussen (3).

The tip of the conjugation process or trichogyne makes contact with the antheridium, and the contents of the antheridium pass over into the oogonium.

Sections through a sorus of sexual organs show a condition very comparable to that figured by Harper (8, Pl. XIX). It would appear that the entry of the male nuclei is a fairly protracted affair. There is a dense massing of the nuclei towards the upper region of the oogonium, and in the fringes of the mass nuclei are seen to fuse in pairs. Pl. XVI, Fig. 1 is drawn from a section which has passed a little to one side of the mass, and shows unfused, fusing, and fused nuclei. The fused nuclei stain more deeply, but this cannot be taken as a criterion. The antheridium and conjugation process contain a deeply staining residue, but no visible nuclei. The ascogenous hyphae are already growing out and contain nuclei.

When the tips of the ascogenous hyphae bend over to initiate the young asci, simultaneous divisions of two nuclei occur, and present an interesting deviation from the course indicated for other Ascomycetes by previous observers. In some of them the nuclei show 7 chromosomes on the spindle in metaphase, and 14 (7 to either pole) in anaphase, and are accordingly in the haploid condition. In others 14 chromosomes are shown in metaphase, and over 20 can be counted on their way to the poles, indicating that in this case the diploid condition has been attained. It is essential to bear in mind that no significance can be attached to a count of 14 chromosomes, since this number occurs alike in the metaphase of a division with 14 chromosomes and in the anaphase of a division with 7. In many nuclei of this size it is impossible to be quite certain of the phase of division.

A fusion of two nuclei in the young ascus is commonly observed. In the figures of the meiotic division the definitive nucleus is observed to be sometimes diploid (Pl. XVI, Figs. 3 and 4) and sometimes tetraploid (Pl. XVI, Figs. 9 and 10). Pl. XVI, Fig. 10 shows fourteen chromosomes (the diploid number) passing to either pole after the reduction division. Pl. XVI, Figs. 5 and 6 show haploid nuclei at the binucleate stage, while Pl. XVI, Fig. 11 shows diploid ones. The upper of the two nuclei in Pl. XVI, Fig. 11 is cut, but the lower one is critical.

It will thus be apparent that if the third division be normal homotype, the resultant spores in an ascus such as that in Pl. XVI, Fig. 11 will be diploid. In all observed cases the spore-nuclei are haploid, and it may further be pointed out that successive generations of the strain show no alteration in chromosome number. Pl. XVI, Fig. 12 presents a feature which is as interesting as it is uncommon in preparations. The nuclei are reorganizing after the third division, and the chromosomes are astonishingly clear. The upper two nuclei are cut, but five of the remaining six show seven chromosomes and a centrosome each. The eighth nucleus is overlaid by its sister-nucleus.

Paired nuclei are commonly seen whenever rapid growth is taking place. They are observed in germ-tubes, unfertilized oogonia, and intact antheridia, in hyphae proceeding from the stalk-cell of the oogonium, and in ascogenous hyphae.

DISCUSSION.

The literature of the cytology of the Ascomycetes is now of considerable bulk. It is not proposed (and it would be neither necessary nor desirable) to go over the whole field in close detail. Too much emphasis cannot be laid on the fact that no claim is made that the state existing in *P. domesticum* is of general occurrence in the Ascomycetes. It is hoped, however, that it may throw light on the conflicting views of the life-history of these plants.

Harper (8) in 1900 figured and described for *P. confluens* the occurrence of successive fusions in the oogonium and in the young ascus. Fraser (5), working on *Humaria rutilans*, reported that in the ascus two of the three divisions are reducing divisions, the chromosome number being halved both in the first and in the third division in the ascus; the first and second divisions constitute a meiotic phase, and the third brings about a shortened second reduction, hence called brachymeiosis.

Claussen, after re-working *P. confluens*, announced in his paper of 1912 (3), 'Eine Brachymeiosis existiert nicht'. The evidence for this categorical statement is purely negative, and seems to be based on an apparent balance of probabilities in the face of a considerable amount of positive evidence of the existence of brachymeiosis. Further, it seems fair to examine the

evidence put forward by Claussen in the light of what has been shown for *P. domesticum*. For *P. confluens* (3) he gives it as his opinion that the haploid number of chromosomes is twelve; but an examination of Pl. VI (loc. cit.) will show that conditions analogous to those described for *P. domesticum* may well exist. In no case after the first division does he show twelve chromosomes going to either pole. Nothing else is decisive. Of Fig. 107 he says 'In der Anaphase wandern 12 Chromosomen nach jedem Pol'. The figure in question shows five going one way and four the other. Fig. 117 shows two nuclei in which the chromosomes are aggregated and two others, one of which shows six (presumably a metaphase), and the other has six passing to either pole.

It must be admitted that it is not easy to formulate homologies with other plants from the interpretation here put forward, but it is submitted that any other would be a severe strain on the facts. It is impossible to demonstrate that one of the two nuclei which fuse in the oogonium is antheridial and the other oogonial. But when the entry of the male nuclei is observed, nuclei are seen fusing in pairs, and there are correlated changes in chromosome number, it is fair to infer that a sexual fusion has taken place; scarcely fair to interpret such fusions as pathological.

Bagchee (1), working on a fungus which he cites as *Pustularia bolarioides*, Ramsb., has demonstrated quite satisfactorily that there is but one reducing division in the ascus of that organism; he has not, apparently, examined the initial stages. Whatever be the result of a single investigation, however complete, it will justify no sweeping generalizations.

It would appear that *P. domesticum* is in a transition stage. The sexual fusion is occasionally omitted, and the omission may be due to a rapid outgrowth of ascogenous hyphae and the passage of haploid nuclei into them. The fusion of two nuclei in the ascus has never been seriously disputed, and there is no evidence in the present case for its omission. As a borderline case it is hoped that *P. domesticum* may effect some liaison.

SUMMARY.

1. In *Pyronema domesticum*, (Sow.) Sacc., after the passage of the ♂ nuclei into the oogonium, a massing of the nuclei occurs, and some of them are observed to fuse in pairs.

2. Nuclei in the diploid and in the haploid condition are seen in the ascogenous hyphae. The haploid number of chromosomes is seven.

3. The definitive nucleus of the ascus is in some cases diploid and in others tetraploid, while the nuclei of the binucleate stage are sometimes diploid and sometimes haploid.

4. The nuclei at the end of the third division are always observed to be haploid, showing that in the case of nuclei which were diploid after meiosis a further reduction has taken place.

I am indebted to the Dixon Fund for the use of a suitable microscope. My thanks are due to Professor Dame Helen Gwynne-Vaughan, under whose direction the work has been carried out, for much kindly help, for considerable searching criticism, and for a judicious tempering of encouragement. I have also to thank Mr. J. Ramsbottom and Mr. B. Barnes for help on many points.

The work has been carried out in the laboratories of the Birkbeck College. The investigation in its later part was made possible by means of a Research Studentship granted by the College.

LITERATURE CITED.

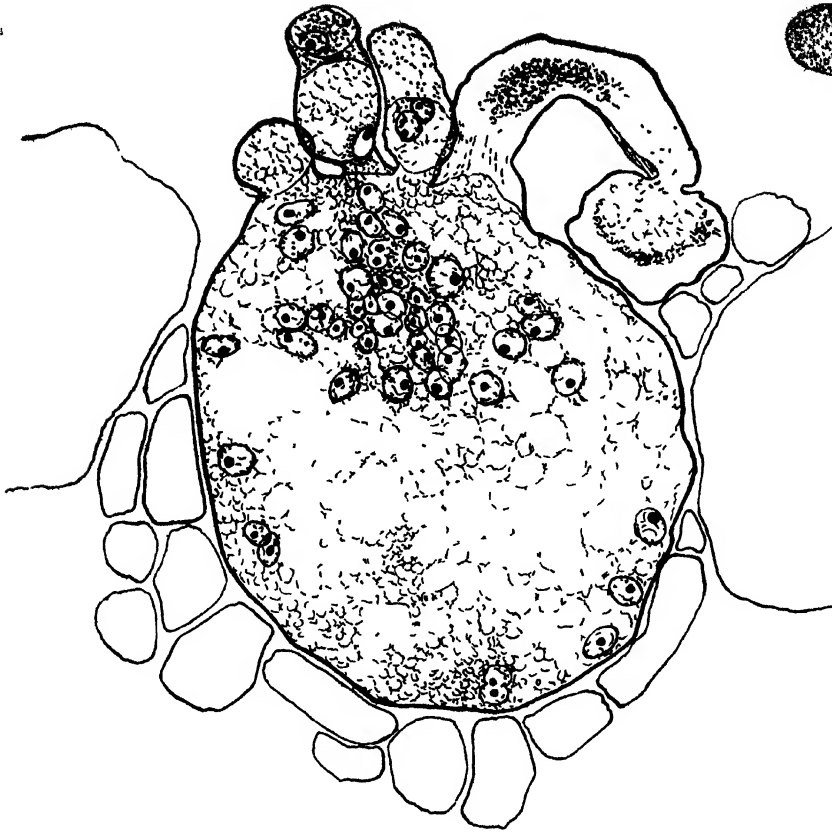
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EXPLANATION OF PLATE XVI.

Illustrating Mr. Geoffrey Tandy's paper on the Cytology of *Pyronema domesticum*, (Sow.) Sacc.

Figures drawn with the aid of a camera lucida. $\times 1900$.

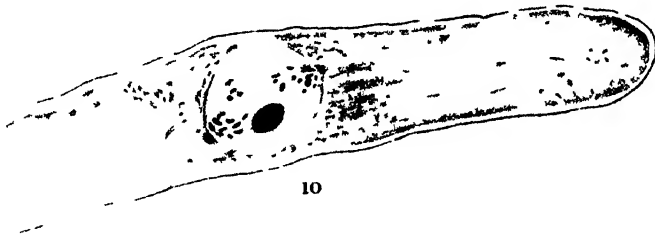
- Fig. 1. Oogonium after the entry of the σ^7 nuclei.
 Fig. 2. Simultaneous divisions of haploid nuclei in ascogenous hypha.
 Fig. 3. 1st division metaphase (diploid nucleus).
 Fig. 4. „ „ anaphase (diploid nucleus).
 Fig. 5. 2nd „ metaphase (haploid).
 Fig. 6. „ „ anaphase and telophase (haploid).
 Fig. 7. 3rd „ metaphases and anaphase (haploid).
 Fig. 8. Simultaneous divisions of diploid nuclei in ascogenous hyphae.
 Fig. 9. 1st division equatorial plate (tetraploid).
 Fig. 10. „ „ anaphase (tetraploid).
 Fig. 11. 2nd „ anaphase (diploid).
 Fig. 12. Reorganization of daughter-nuclei of the 3rd division.



1



2

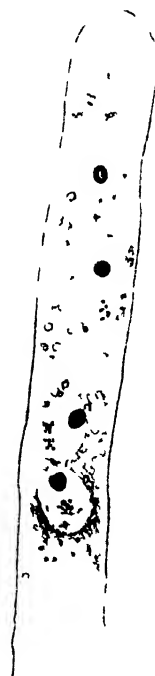


10



12.

TANDY-PYRONOMA



Field Observations on Starch Production in the Leaves of the Potato.

BY

E. J. MASKELL

(Imperial College of Science and Technology, London, and Rothamsted Experimental Station).

With two Figures in the Text.

THE effect upon yield of crop plants of such changes in their environment as can be brought about by cultivation or manuring offers many problems to the plant physiologist. Among the most interesting of these problems is that of the analysis of the observed effect upon yield in terms of the effect upon the various processes at work in the plant. For the purpose of this inquiry, it is a great advantage if the field experiment can be arranged so as to furnish not only data for yield but also data concerning those aspects of plant behaviour in terms of which the analysis of yield may be attempted. Manurial experiments offer very favourable material for studies of this type, and the present paper is concerned with some preliminary work on one selected aspect of the behaviour of potatoes in a manurial experiment which has been carried out for the past four years at Rothamsted by Mr. T. Eden.

The work has covered so far only the later period of growth of the plants, and a discussion of the results in relation to quantity and quality of yield is not as yet possible. The chief interest of the present paper lies rather in the statistical analysis of the experimental results and in the evidence which the results supply of the usefulness for field studies of the method employed. The bearing of the work upon the problems of yield will, however, become clearer if some account is first given of the nature of the potato experiment and of the yield results which have so far been obtained. For this summary the writer is indebted to Mr. T. Eden.¹

The experiment is a comparison of the effect upon the yield of potatoes of potash, supplied in three different forms. The four manurial treatments involved are : (1) Control, i. e. no potash ; (2) potash as potassium sulphate

¹ See also Journ. Ministry of Agriculture, Jan., 1926. Report of Potato Conference.

(47·71 per cent. K_2O); (3) potash as potassium chloride (52·11 per cent. K_2O); (4) potash as 'potash manure salts' (P.M.S.) (27·65 per cent. K_2O). The quantities of the three potash manures are so adjusted that all plots receive an amount of potassium per acre equal to that in 2 cwt. of potassium sulphate. The basal manuring for all plots is 2 cwt. per acre of sulphate of ammonia and 6 cwt. per acre of superphosphate. The variety of potato employed is Kerr's Pink (Scotch seed).

In considering the yield results so far obtained we may distinguish two main questions: (1) the effect of 'potash' as against 'no potash'; (2) the effect, within the group of potash manures, of the other substances associated with the potash. The results show a significant superiority of 'potash' over 'no potash' in yield of tubers, the magnitude of the potash effect being a function of season. As between the three forms of potash there is no significant difference that is consistent from year to year, but there seems to be a significant differential response to season, the P.M.S. plots giving relatively low yields in some years. The starch content of the tubers is, however, not the same for all the potash manures. The values vary with manurial treatment as well as with season, the averages for three years being K_2SO_4 62·17 per cent., KCl 59·72 per cent., P.M.S. 57·13 per cent., 'no potash' 58·07 per cent. When this effect upon starch content is considered in relation to yield the differences between the various treatments become significant, the mean value of starch per acre for the three years being K_2SO_4 1·3243, KCl 1·2180, P.M.S. 0·9643, 'no potash' 0·8377.

It will be seen that the relation of these manurial treatments to the production of starch by the potato plant is of considerable interest, and a study of the behaviour of the leaves as regards production and removal of starch seemed desirable.

For the purpose of this field study on the experimental plots some simple method which would allow of simultaneous observations on all four plots was required, and Sachs's 'iodine' method seemed likely to be useful. This method has been used as a quantitative one by Ursprung¹ in a study of the influence of light of different wave-lengths upon starch formation, and the method which he employed, of grading the amount of starch formed by comparing the colour developed in the iodine test with a standard scale of colour tones, has been adopted here. Preliminary trials showed that matching of the tones was easier and more accurate if a scale containing some violet colour was used rather than a scale of neutral or carbon grey. The scale finally selected was No. 59ⁱⁱⁱⁱ in Ridgway's 'Colour Standards and Nomenclature' (Washington, D.C., 1912). The scale in question consists of nine tones; a central colour, No. 5 in the scale, being modified by progressive increments of white (tones 4, 3, 2, 1) or of black (tones 6, 7, 8, 9); the

¹ Ursprung, A. : Ber. d. Deutsch. Bot. Ges., Bd. xxxv, p. 44, 1917.

increments of white being 9.5 per cent., 22.5 per cent., 45 per cent., and 100 per cent., and of black being 45 per cent., 70.5 per cent., 87.5 per cent., and 100 per cent. The central tone is a broken colour containing 95.5 per cent. of neutral grey and 4.5 per cent. of violet (wave-length 410). The neutral grey is equivalent to a colour-wheel mixture of black 79 per cent., white 21 per cent. The colour-wheel analysis of the scale from Ridgway's data is shown in Table I.

TABLE I.

Analysis of Colour Tone Scale, Ridgway 59''''.

<i>Tone No.</i>	<i>White %.</i>	<i>Black %.</i>	<i>Violet %.</i>
1.	100	—	—
2.	55.77	41.75	2.48
3.	37.67	58.84	3.49
4.	27.22	68.71	4.07
5.	19.58	75.92	4.50
6.	10.77	86.75	2.48
7.	5.78	92.89	1.33
8.	2.45	96.99	0.56
9.	—	100	—

The colour intensity developed by the iodine test in any leaflet may therefore be expressed as a number on the scale of tones, or as a percentage of black: and, similarly, differences in colour intensity between different leaflets may also be expressed in either units. It will be seen, however, that successive increments on the tone scale from 1 to 9 correspond to progressively smaller increments of percentage of black. Thus in estimating starch production by differences in colour intensity shown by the iodine test much depends on the scale in which the results of the colour grading are expressed. In the absence of precise information as to the relation between starch content and the colour tone shown by the iodine test (information which it is hoped to obtain next year), the results presented in this paper have been calculated on both bases, greater weight being assigned to the tone scale basis as representing the primary data. There is, moreover, some evidence which suggests that the tone scale does roughly correspond with starch content over the greater part of its range.

This evidence is derived from tests which were made with a series of solutions of soluble starch in distilled water, ranging in concentration from 0.0065 per cent. to 1.664 per cent. One c.c. of each solution was run evenly on to a No. 1 Whatman filter-paper of 9 cm. diameter; two filter-papers being prepared in this way from each solution. The eighteen filter-papers were then dried in an oven at 90° C. for three hours. Four squares of about 2 cm. diameter were cut from each, the whole set stained in iodine solution as in Sachs's test, washed in running water till no trace of brown discoloration due to iodine remained on the filter-paper, spread on

the surface of a white plate, and compared with Ridgway's colour scale 59^{III}.

The average colour value for the eight squares prepared from each solution is given in Table II.

TABLE II.

<i>Starch Solution.</i>	<i>Mean Colour Tone.</i>
%.	
0.0065	< 1.25
0.0130	< 1.25
0.0260	1.35
0.0520	1.65
0.1040	2.25
0.2080	3.20
0.4160	4.65
0.8320	7.10
1.6640	8.50

The increment of starch content corresponding to a single step in the tone scale increases as we go up the tone scale: this increase is relatively slow between tones 1 to 7, but very rapid after that. In the experiments on the potato leaves the colour intensity observed rarely exceeded tone No. 7, and, in view of the relatively small departure from a linear relation between tone scale and starch concentration over the range 1-7, that scale may be considered the best simple approximation for the estimate of changes in starch content in the experimental leaflets.¹

Experimental Procedure.

The object in view was to obtain, at different periods during growth, estimates of the net amount of starch produced, during a specified time interval, by leaves of plants on the experimental plots. This net starch production was estimated as the difference between the colour value developed in the iodine test by a leaflet exposed to light on the plant for three hours and the colour value of *its opposite leaflet* which had remained covered during the three hours. On the assumption that loss of starch due to translocation and respiration proceeds as rapidly in the covered leaflet as in the exposed, the difference between the two should be a measure of net starch production and would correspond to an estimate of real assimilation. In as far as the rate of loss of starch by translocation, or of carbohydrate which might other-

¹ It may be of interest to note the amounts of starch required on the basis of the above figures to give the colour tones observed in the case of the potato leaflets. These are, for the potassium sulphate plot :

Mean colour tone,	{ covered leaf 1.78 \equiv 0.511 mg. starch per 50 sq. cm. filter-paper.			
	{ exposed leaf 4.5 \equiv 3.270	"	"	"
Difference (= starch production)	\equiv 2.759	"	"	"

wise have become starch, is a function of carbohydrate concentration in the leaflet, this rate of loss should be somewhat greater in the exposed leaf, and the estimate of net starch production will therefore be a minimum one.

The procedure in making the observations was as follows: One plot of each treatment was chosen from the field experiment, which was in the form of a Latin square, 4×4 , each plot $\frac{1}{16}$ acre in area. The four plots chosen formed one row of the experiment and had been arranged at random within the row. Between 5 and 6 p.m. on the evening before observations were to be made a pair of opposite leaflets on each of twelve plants on each of the four plots were covered by black paper envelopes, these being secured, without damage to the petiole, by paper clips partially closing the opening by which the envelope was slipped on to the leaflet.

At about 9.30 a.m. on the following day the black envelope was removed from one leaflet of each of six plants on each plot. Three hours later the six leaflets thus exposed were, together with their opposite (covered) leaflets, removed from the plant and taken to the field laboratory. At about the same time envelopes were removed from one leaflet of each of the six remaining marked plants on each plot, and again three hours later both the exposed and the covered leaflets were removed and taken to the field laboratory. On being brought into the field laboratory the leaflets were at once boiled for ten minutes in water, decolorized in warm alcohol, stained in a solution of iodine in potassium iodide, and then washed in tap-water until no traces of free iodine remained. They were then spread on a white enamel plate and their colour compared with the Ridgway scale. Up to this point each of the samples of six leaflets was treated as a unit, as it was not found practicable to keep the exposed and covered leaflets in their original pairs. The colour tone of each leaflet was estimated separately.

The leaflets used were in all cases a pair of opposite leaflets next the terminal leaflet on the fourth leaf from the apex of a haulm, since a preliminary survey had shown that these were usually of a convenient size and had a fairly uniform distribution of starch. Older leaflets were, especially on the potash-starved plot, liable to marked patchiness in starch distribution. Throughout, only leaves which were green and apparently healthy were used.

Experimental Results.

Owing to the pressure of other work it was not possible to begin the observations until September. The plots had been planted on April 30, and the plants had appeared above ground about June 15. By the beginning of September growth of the haulms had almost ceased, and the observations relate therefore to the final stages of the vegetative life-cycle. The appearance of the plants at this time may be summarized as follows: (a) Potassium sulphate plot. Plants of a bushy habit, foliage a healthy green, but the

older leaves beginning to yellow and ripen off during the month. (b) Potassium chloride plot. Plants somewhat taller but less bushy than on the sulphate plot: foliage green, but the older leaves yellowing earlier than on the sulphate plot. (c) P.M.S. plot (30 per cent. potash manure salts). Plants similar to those on the potassium chloride plot, but the older leaves yellowing somewhat earlier. (d) No potash plot. Plants stunted and most of

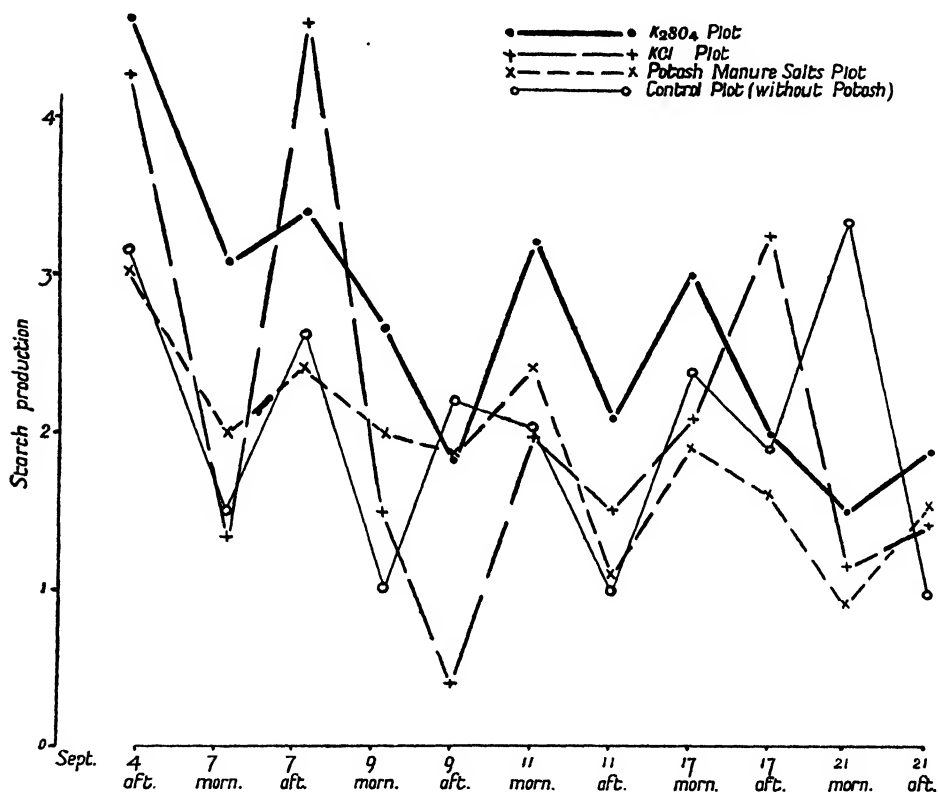


FIG. 1.

the older leaves discoloured with copper-coloured patches (often areas of local death).

The results of all the experiments in which complete data from all four plots were secured are given in Table III, and the values for starch production are shown in Fig. 1. The figures in the table are the means of the tone values for the six leaflets of each sample. The values for starch production are the differences between the values for the exposed and covered leaflets and refer to a period of three hours. (The first experiment lasted three hours and twenty minutes, and the figures for starch production have been corrected for three hours.) The mean values for each occasion irrespective

TABLE III.
Starch Production in Potato Leaflets (Tone Scale Values).

Treatment →		K ₂ SO ₄			No Potash.			P.M.S.			KCl.			Mean.		
Date.	Period.	Exposed.	Covered.	Starch Production.	Exposed.	Covered.	Starch Production.	Exposed.	Covered.	Starch Production.	Exposed.	Covered.	Starch Production.	Exposed.	Covered.	Starch Production.
Sept. 4	1 ft.	7.50	2.37	4.62	6.83	3.33	3.15	7.12	3.75	3.03	6.88	2.12	4.28	7.083	2.893	3.770
" 7	Morn.	5.16	2.08	3.08	4.83	3.33	1.50	4.16	2.16	2.00	3.33	2.00	1.33	4.370	2.393	1.977
" 7	Aft.	4.58	1.16	3.42	4.45	1.83	2.62	4.16	1.75	2.41	6.00	1.42	4.58	4.798	1.540	3.258
" 9	Morn.	4.25	1.58	2.67	3.75	2.75	1.00	4.33	2.33	2.00	3.92	2.41	1.51	4.063	2.268	1.795
" 9	Aft.	3.17	1.33	1.84	4.41	2.21	2.20	4.58	2.71	1.87	3.17	2.75	0.42	3.833	2.250	1.583
" 11	Morn.	3.17	1.30	3.20	4.75	2.70	2.05	5.42	3.0	2.42	5.10	3.10	2.00	4.943	2.525	2.418
" 11	Aft.	3.50	1.41	2.09	3.58	2.58	1.00	3.67	2.58	1.09	3.60	2.08	1.52	3.588	2.163	1.425
" 17	Morn.	4.17	1.17	3.00	3.82	1.42	2.40	4.0	2.08	1.92	3.82	1.75	2.07	3.953	1.605	3.348
" 17	Aft.	3.82	1.82	2.00	3.33	1.43	1.90	4.58	1.30	1.62	4.58	1.33	3.25	3.063	1.470	2.128
" 21	Morn.	4.08	2.58	1.50	5.67	2.33	3.34	2.92	3.67	0.91	4.08	2.92	1.16	4.603	2.875	1.765
" 21	Aft.	4.75	2.83	1.92	4.90	3.92	0.93	3.30	1.75	1.55	3.58	2.17	1.41	4.133	2.668	1.465
Mean →		4.498	1.785	2.667	4.575	2.530	2.013	4.385	2.462	1.893	4.369	2.186	2.139	4.457	2.241	2.178

of treatment are shown in three columns at the extreme right, and the mean values for each treatment irrespective of 'occasion' in the lowest row of the table.

It will be seen that in no case has starch quite disappeared from the covered leaflets (the values for the average tone being in all cases greater than 1). Comparison of the values for starch production depends, therefore, very largely on the relation between increments in the tone scale and increment in starch content. We have seen that the tone scale forms at least an approximation to the required scale, and the preliminary analysis of the results will therefore be carried out on the figures of Table III. The general results will, however, be checked by an analysis of the results on the basis of percentage of black scale (see p. 340).

Statistical Analysis of the Variation in Starch Production.

The data for starch production form a 4×11 table (four manurial treatments compared on eleven 'occasions'), and for data of this form R. A. Fisher has developed a very valuable method of analysis—the analysis of variance.¹ The method enables one to estimate the amount and the significance of the contribution made by 'manurial treatment' on the one hand and by 'occasion' on the other to the total variation of the set of observations. This analysis is shown in Table IV.

TABLE IV.

Analysis of Variance of Starch Production.

	<i>Sum of Squares.</i>	<i>Degrees of Freedom.</i>	<i>Variance.</i>	<i>Log_e Variance.</i>
Total	40·5219	43		
Manuring	3·84495	3	1·28165	0·24818
Occasion	22·42560	10	2·24256	0·80762
Differential	14·25135	30	0·475045	0·255655

The total variation is represented by 40·5219, which is the sum of the squares of the deviations of each of the forty-four observed values from their mean. The contribution to this total made by manuring, 3·84495, represents eleven times the sum of the squares of the deviations of the mean of each manurial treatment from the general mean. The number of degrees of freedom available for this contribution is three (one less than the number of manurial treatments). Similarly, the sum of squares due to 'occasion' is four times the sum of the squares of the deviations of the mean of each of the eleven 'occasions' from the general mean, and ten degrees of freedom are available. The remaining fraction of the total sum of squares, 14·25135 ($= 40·5219 - 3·84495 - 22·42560$), represents differential behaviour of the

¹ R. A. Fisher: *Statistical Methods for Research Workers*, London and Edinburgh, 1925.

different manurial treatments on different occasions, and to it are assigned the remaining thirty degrees of freedom. The mean square or variance (= sum of squares/degrees of freedom) is shown in the third column, and it will be seen that the variances due to 'occasion' and to manuring are much greater than that due to differential response. It remains to test whether the relative magnitudes of these variances indicate a significant effect of 'occasion' independent of manuring, and of manuring independent of 'occasion', upon starch production, or whether a chance combination of differential effects could have given rise to the observed figures. The standard of comparison developed by Fisher for this purpose is half the difference between the natural logarithms of the variances to be compared. For the comparison between the variances due to manuring and to differential effects, this value, Z , is equal to 0.49621; for the similar comparison between 'occasion' and differential effects Z is equal to 0.77598. For the first case, the value of Z required for $P = 0.05$, i. e. for the probability that a difference as great as that observed shall occur by chance not more than once in twenty trials, is 0.5364. In the second case the value of Z required is lower; since ten instead of three degrees of freedom are available for estimating the larger variance, the value of Z required is 0.4055.

The contribution made by manuring just fails to reach a significance of $P = 0.05$, while the contribution of 'occasion' is undoubtedly significant. The relatively small contribution made by manuring independent of 'occasion' to the total variation is due, as the lower row of Table III shows, to the fact that the two potash manures containing chlorides have hardly any effect on the mean starch production. The potassium sulphate plot alone shows any superiority over the potash-starved plot. This single manurial effect is swamped in the general analysis by the absence of effect from the other two manurial treatments. For an estimate of the significance of this effect we may calculate from the differential variance the standard deviation of a comparison between the means of any two manurial treatments.

$$\sigma = \sqrt{\frac{0.475045 \times 2}{11}} = 0.29389.$$

$$2 \sigma = 0.58778.$$

A difference between means exceeding 0.58778 may therefore be regarded as significant. The observed differences are $K_2SO_4 - KCl = 0.528$, - no potash = 0.654, - P.M.S. = 0.774, so that the K_2SO_4 plot is definitely superior to the 'no potash' and the P.M.S. plot, but there is no significant difference between KCl, 'no potash', and P.M.S.

In the above analysis the variance due to differential response has in effect been used as an estimate of random variance. It is *a priori* probable that the differently manured plots will respond differently on different occasions, but to estimate the importance of this factor we require an estimate of

the random variance due to sampling. From the data for the individual leaflet we may make an estimate of the random variance to be expected in a set of observations derived from samples of six leaflets. The essential data are given in Table V.

TABLE V.

<i>Variance of Individual Leaflets.</i>	<i>Variance of the Difference =</i>		<i>Variance of</i>	
<i>Plot.</i>	<i>Exposed.</i>	<i>Covered.</i>	<i>Starch Production.</i>	<i>Mean Starch Production.</i>
K ₂ SO ₄	2.076	1.029	3.105	0.5175
No potash	3.199	2.416	5.615	0.9358
P.M.S.	2.051	2.012	4.063	0.6772
KCl	1.851	1.229	3.080	0.5133
Mean	2.294	1.6715	3.966	0.6611

It will be seen that, assuming no correlation between the covered and the exposed leaflets, the random variance calculated, 0.6611, is greater than the differential variance observed (Table IV). There is, however, a strong correlation between paired leaflets which the above calculation neglects. Direct calculation of the variance of the difference between paired leaflets, one covered and one exposed, is not possible, because, owing to the large number involved, the leaflets could not be kept in their pairs during boiling and decolorizing for the starch test. There are data, however, for six pairs of leaflets from each plot on September 10, from which the following coefficients of correlation between paired leaflets are calculated: K₂SO₄ plot, $r = +0.8094$; no potash plot, $r = +0.8274$; P.M.S. plot, $r = +0.8250$; KCl plot, $r = +0.8163$. For the whole set, twenty-four pairs, $r = +0.9348$. We may use the lower figure +0.8 for an approximate estimate of the variance of the difference between paired leaflets.

From the relation $\sigma^2_{A-B} = \sigma^2_A + \sigma^2_B - 2\sigma_A\sigma_B r_{AB}$, where A and B refer to the exposed and covered leaflets, if $r_{AB} = +0.8$, then $\sigma^2_{A-B} = 0.1295$. This is probably too low an estimate, but even if it were doubled the variance due to differential effects would still remain insignificantly greater. There is some evidence, therefore, for a significant differential effect of manure and 'occasion' upon starch production.

Returning now to the interpretation of the mean values for starch production for the different treatments, it is evident from the bottom row of Table III that the superior starch production of the potassium sulphate plot is associated rather with a decrease in the mean value of the covered leaflets than with an increase in the mean value of the exposed leaflets. The mean values for the exposed leaflets are indeed almost the same for all the plots. The data suggest—and the suggestion is confirmed, at least for the first half of the month, by the results of a few direct experiments on

translocation—that the rate of translocation of starch from the leaves is greater with the potassium sulphate manuring, less with the KCl and P.M.S. manuring, and least in the absence of potash. From Table III a comparison of the mean values for the covered leaflets at 12.30 (*circa*) and at 3.30 (*circa*) shows that by noon the K_2SO_4 leaves have already a low starch content and show thereafter little further change. The two chloride plots, while relatively high at noon, fall in the afternoon towards the level of the K_2SO_4 plots, while the potash-starved plot, which is as high as these at noon, falls but slowly during the next three hours.

Average Covered Leaf Values.

	K_2SO_4	No potash.	P.M.S.	KCl.
12.30 p.m.	1.742	2.506	2.628	2.432
3.30 p.m.	1.712	2.396	2.018	1.830

Again, the average of a number of samples of untreated leaflets taken about 9.30 a.m. shows the potash-starved plot still high in starch (6.08), while the other plots are low (KCl 5.08, P.M.S. 4.85, K_2SO_4 4.7). The mean of a number of similar samples taken at 5 p.m. shows the plots in exactly the reverse order, though the differences between plot and plot are smaller.

The association of the higher figure for mean starch production with the lower mean starch content of the covered leaflet thus suggests three possibilities. (1) That during the three hours' exposure all leaflets, irrespective of treatment, can reach, but cannot exceed, a certain limit of starch content. (2) That the rate of starch production is lower the greater the amount of starch already in the leaf. (3) That within the manurial plots under observation a low rate of starch removal is associated with, though not of necessity causally related to, a low rate of starch production, both phenomena being due to an impaired protoplasmic mechanism. On either of the first two suppositions the superiority in starch production shown by the potassium sulphate plot would be ascribed to a superior rate of translocation of starch and would presumably not have been shown had all the leaflets been completely destarched before exposure. The first possibility is, however, excluded by an inspection of the mean values for the exposed leaflets on each of the eleven 'occasions'. They are far from constant, their standard deviation being greater than that for the covered leaflets, 0.9781 as against 0.5106. If the second supposition is correct there should be a strong negative correlation between the mean values for starch production on each occasion and the corresponding values for the covered leaflets. The correlation coefficients are (where starch production = 1, exposed leaflet = 2, covered leaflet = 3):

$$r_{12} = +0.7356$$

$$r_{13} = -0.0992.$$

The correlation with the covered leaflet is indeed negative, but is insignificantly small. The amount of starch left in the leaf thus contributes little to the variation in starch production on different occasions. This factor can therefore be of small importance in accounting for the mean difference in starch production between treatments. This becomes quite clear if, using the regression coefficient for the equation between starch production and covered leaflet value, we calculate from the mean covered leaflet value for each treatment the expected difference in mean starch production between treatments. Thus we have

	<i>Expected.</i>	<i>Observed.</i>	<i>Difference.</i>
K ₂ SO ₄ — KCl =	0.0584	0.528	0.4696
K ₂ SO ₄ — no potash =	0.1085	0.654	0.5455
K ₂ SO ₄ — P.M.S. =	0.0986	0.774	0.6754
Mean K ₂ SO ₄ — rest =	0.0892	0.652	0.5628

Thus the superior starch production of the K₂SO₄ plot, while apparently associated with, is not wholly due to a more rapid rate of translocation of starch from the leaf. The mean difference in rate of starch production may well be due to differences in the efficiency of the photosynthetic mechanism.

Analysis of the Variance due to 'Occasion'.

In the analysis of variance of starch production ten degrees of freedom were assigned to 'occasion'. It will be interesting to see whether the greater part of this variance can be ascribed to the varying intensity of one or two main factors such as might influence rates of carbon assimilation. Two factors suggest themselves: (1) intensity of radiation, (2) age, since we are dealing with the phase of growth in which 'ripening off' is beginning. Radiation data are available from the Callendar Recorder charts, and have been calculated as average intensity of total radiation during the period of exposure of the leaflets.¹ Age may be reckoned in days, the first day of the experiments, September 4, counting as one (Fig. 2). The correlations between these factors and the mean starch production (irrespective of treatment) are as follows (1 = starch production, 2 = radiation, 3 = age):

$$\begin{array}{ll}
 r_{12} = +0.23629 & r_{12.3} = +0.58363 \\
 r_{13} = -0.47837 & r_{13.2} = -0.67927 \\
 r_{23} = +0.45828.
 \end{array}$$

It will be seen that the partial correlation coefficients are of the sign and approximately of the order that would be expected if we were dealing with carbon assimilation. It remains to estimate their significance. The

¹ The values given for radiation in Fig. 2 represent millimetres on the Callendar Recorder charts. One millimetre corresponds to 0.0092 calorie per cm.² per min.

calculation involves the fitting of two constants derived from the data, hence the number of degrees of freedom remaining is 8 ($10 - 2$). For this number of degrees of freedom the levels of significance are: ¹

$$\text{for } P = 0.1 \quad r = 0.5494$$

$$P = 0.05 \quad r = 0.6319.$$

The negative correlation with age is therefore quite significant, while the

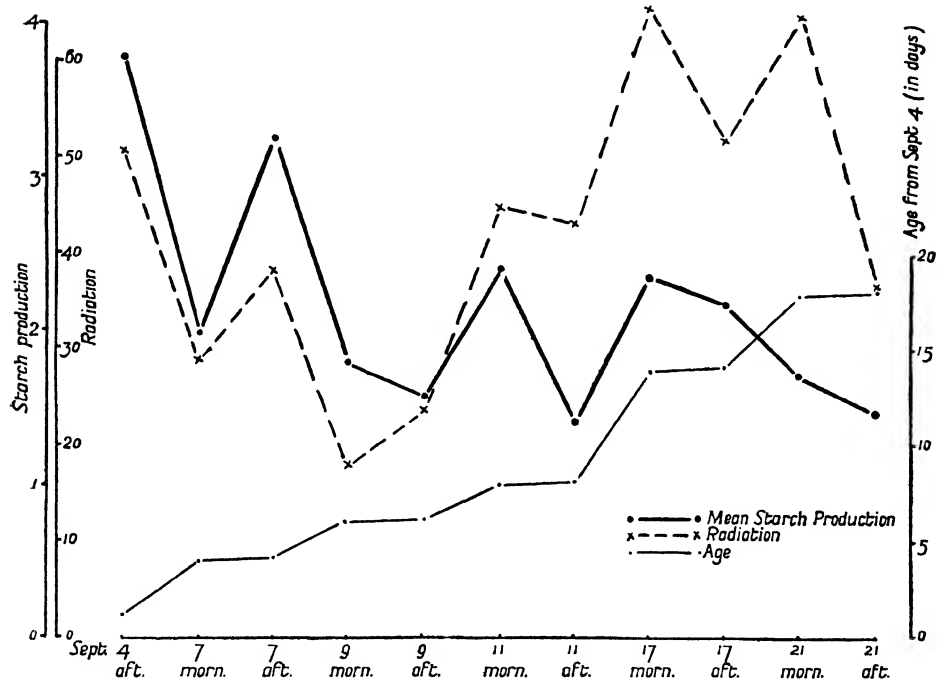


FIG. 2. Graph showing the relationship between mean starch production, radiation, and age of leaf for the period Sept. 4 to 21.

positive correlation with radiation is, owing to the small number of degrees of freedom available, less certain (P lies between 0.1 and 0.05). The two factors together, however, account for more than 60 per cent. of the total sum of squares due to 'occasion', as Table VI shows.

TABLE VI.

Analysis of Variance due to 'Occasion'.

	Sum of Squares.	Degrees of Freedom.	Variance.	Log ϵ Variance.
Total	22.42560	10	2.24256	0.80762
Fit to regression on radiation and age	14.02060	2	7.01030	1.94738
Deviation	8.40504	8	1.5063	0.40954

¹ Fisher, loc. cit., Table V A.

Comparing the variance due to radiation and age with the remainder, we have $Z = 0.76892$. For $P = 0.05$ Z should be 0.7475 ,¹ so that the contribution made by these two factors to the total variance is significant.

Differential Response of Different Treatments.

In view of the suggestion (see p. 336) of a significant differential response of manurial treatment and 'occasion' the correlations with radiation and age for each plot separately will be of interest.

These are (where 1 = starch production, 2 = radiation, 3 = age) :

K_2SO_4	$r_{12.3} = +0.47388$	P.M.S.	$r_{12.3} = +0.18442$
	$r_{13.2} = -0.73641$		$r_{13.2} = -0.64938$
No potash	$r_{12.3} = +0.62740$	KCl	$r_{12.3} = +0.46645$
	$r_{13.2} = -0.39012$		$r_{13.2} = -0.50104$

The correlations which reach a level of significance, $P = 0.05$, are underlined. It is interesting to note that the plot deficient in potash gives the highest positive correlation with radiation and the lowest negative correlation with age. The values for starch production (Table III and Fig. 1) show that the superiority of the potassium sulphate plot over the 'no potash' plot diminishes as radiation increases or as the plants age. So far as concerns radiation, this effect is in the direction that might be expected if we were dealing with rates of carbon assimilation, since there is evidence from the work of Briggs² that deficiency in mineral salts diminishes the efficiency of the photochemical and chemical phases of photosynthesis, and the effect of this diminished efficiency upon the rate of photosynthesis under natural conditions of carbon dioxide supply will be greater the lower the intensity of radiation.

Analysis of the Experimental Results on the Percentage of Black Scale.

So far, the analysis of the results has been made on the basis of the tone scale used in grading the leaflets. Adjusting the individual observations to the scale of percentage of black, we have the results summarized in Table VII.

As will be seen from the analysis of variance, the effect of manuring and of 'occasion' are both significant, and while the mean difference in starch production between the three plots, 'no potash', P.M.S., and KCl, remains insignificant, the superiority of the K_2SO_4 plot is more marked than before. Again, the mean values for the exposed leaflets vary very little from plot to plot, so that the greater starch production of the K_2SO_4 plot depends on the much lower mean value of its covered leaflets. The correlations between

¹ Fisher, loc. cit., Table V A.

² G. E. Briggs: Proc. Roy. Soc., B., vol. xciv, p. 28, 1922.

mean starch production on each occasion and mean values for the covered and exposed leaflets are as follows (where 1 = starch production, 2 = exposed leaflet, 3 = covered leaflet): $r_{12} = +0.44146$, $r_{13} = -0.59863$.

TABLE VII.
Scale of Percentages of Black.

			<i>Mean of all Four Treatments.</i>		
<i>Date.</i>			<i>Exposed.</i>	<i>Covered.</i>	<i>Starch Production.</i>
Sept.	4	Aft.	92.69	45.48	42.5
"	7	Morn.	70.10	39.95	30.15
"		Aft.	74.84	22.35	52.49
"	9	Morn.	66.50	42.24	24.26
"		Aft.	63.67	38.18	25.49
"	11	Morn.	74.96	42.35	32.62
"		Aft.	59.02	36.18	22.84
"	17	Morn.	66.31	25.09	41.22
"		Aft.	61.88	21.34	40.54
"	21	Morn.	69.53	45.08	24.45
"		Aft.	65.36	42.66	22.70

Mean of the Eleven Values for each Treatment.

	<i>Exposed.</i>	<i>Covered.</i>	<i>Starch Production.</i>
K ₂ SO ₄	70.47	25.56	44.398
No potash	70.32	41.26	28.761
P.M.S.	69.19	41.806	24.014
KCl	68.149	37.157	30.457

Analysis of Variance.

	<i>Sum of Squares.</i>	<i>Degrees of Freedom.</i>	<i>Variance.</i>	<i>Log_e Variance.</i>
Total	8798.42	43		
Manuring	2086.62	3	695.54	6.55469
Occasion	4066.80	10	406.68	6.00803
Differential	2645.00	30	88.17	4.47926

Z for 'manuring' = 1.03772.

For $P = 0.05$ Z is 0.5364.

Z for 'occasion' = 0.76439.

For $P = 0.05$ Z is 0.4055.

2 σ for a comparison between means of two manural treatments = 8.007.

The negative correlation between the value for the covered leaflet and starch production is large enough to account for a considerable portion of the observed difference in starch production between different treatments. The following table shows the observed difference between the K₂SO₄ plot and the other three plots, the difference to be expected from the mean values of the covered leaflets for each treatment, and also the remainder.

<i>Plots.</i>	<i>Observed Difference.</i>	<i>Expected Difference.</i>	<i>Remainder.</i>
K ₂ SO ₄ — KCl	13.94	7.67	6.27
K ₂ SO ₄ — no potash	16.64	10.38	6.26
K ₂ SO ₄ — P.M.S.	17.38	10.74	6.64
Mean			6.39

Thus the individual differences between plots remaining after this allowance is made amount to little more than 1.5 times their standard

deviation, and are not significant. The mean difference between the K_2SO_4 plot and the other three is, however, still just significant.

As before, the variance due to 'occasion' can be partly ascribed to varying intensity of radiation and to ageing of the leaflets. The correlation coefficients are of the same sign as before, but of a somewhat smaller order, and do not attain the required level of significance.

$$r_{12\cdot3} = +0.5075 \qquad r_{13\cdot2} = -0.50379.$$

The differential response of the K_2SO_4 and the 'no potash' plots is again evident, and is of the same nature as before.

$$\begin{array}{ll} K_2SO_4 \ r_{12\cdot3} = +0.42005 & \text{No potash } r_{12\cdot3} = +0.64876 \\ r_{13\cdot2} = -0.64224 & r_{13\cdot2} = -0.30071 \end{array}$$

Thus the main features shown by the analysis of the results on the scale of tone values are again found on the scale of percentage of black. We may, however, still keeping to the percentage of black scale for the exposed and covered leaflets, attempt the elimination of the effect of variations in the covered leaflet values by calculating starch production as a percentage of the total possible increase in percentage of black, i.e. as $\frac{100 \times (\text{Exposed} - \text{Covered})}{100 - \text{Covered}}$. On this basis the mean values for 'occasion' and for treatment are as in Table VIII.

The variances due to 'occasion' and to manuring are both significantly greater than the differential variance, and the mean difference between the K_2SO_4 plot and each of the others is also significant. That the method of calculation has to a large extent eliminated the effect of variation in covered leaflet values upon mean starch production is shown by the correlation coefficients (where 1 = mean starch production, 2 = exposed leaflet, 3 = covered leaflet):

$$r_{12} = +0.7863 \qquad r_{13} = -0.1611.$$

The small negative correlation which still remains between covered leaflet value and starch production does not appreciably affect the comparison of the means of manurial treatments, as the following calculation of differences not due to variation in mean covered leaflet value shows:

$$\begin{array}{lll} K_2SO_4 - \text{no potash} & = & 8.054 \text{ instead of } 10.58 \\ K_2SO_4 - \text{P.M.S.} & = & 9.596 \quad \text{,,} \quad 12.21 \\ K_2SO_4 - \text{KCl.} & = & 11.196 \quad \text{,,} \quad 13.06 \end{array}$$

The contributions of radiation and age to the variance due to 'occasion' are now greater than before (1 = starch production, 2 = radiation, 3 = age):

$$^1 r_{12\cdot3} = +0.66172 \qquad r_{13\cdot2} = -0.69611,$$

both correlations being now statistically significant.

The differential response of the K_2SO_4 and 'no potash' plots to radiation and age is of the same order as before :

$$\begin{array}{ll} K_2SO_4 \ r_{12.3} = + 0.47521 & \text{No potash } r_{12.3} = + 0.72587 \\ r_{13.2} = - 0.70971 & r_{12.2} = - 0.67453 \end{array}$$

TABLE VIII.

Starch Production as a Percentage of the Total Possible Increase in Percentage of Black of Scale.

Date.		Mean of all Treatments.	Mean of each Treatment.			
Sept.	4 Aft.	77.33	K_2SO_4 .	No potash.	P.M.S.	KCl.
"	7 Morn.	50.03	59.21	48.63	47.00	46.15
	Aft.	66.48				
"	9 Morn.	40.88				
	Aft.	40.10				
"	11 Morn.	55.30				
	Aft.	34.18				
"	17 Morn.	53.60				
	Aft.	51.50				
"	21 Morn.	44.10				
	21 Aft.	39.43				

Analysis of Variance.				
	Sum of Squares.	Degrees of Freedom.	Variance.	Log. Variance.
Total	10802.06	43		
Manuring	1213.08	3	404.36	6.00230
Occasion	6556.84	10	655.684	6.48568
Differential	3032.14	30	101.071	4.61579

$$\begin{array}{ll} Z \text{ for 'manuring'} = 0.69325. & \text{For } P = 0.05 \text{ } Z \text{ is } 0.5364. \\ Z \text{ for 'occasion'} = 0.93495. & \text{For } P = 0.05 \text{ } Z \text{ is } 0.4055. \\ 2\sigma \text{ for a comparison between means of two treatments} = 8.5742. \end{array}$$

CONCLUSIONS.

It thus appears that in healthy leaflets of potato plants during the later stages of growth the rate of starch production as measured by Sachs's iodine test varies significantly with variations in potash manuring and with certain periodic factors, of which intensity of solar radiation and age have been shown to be important. During this period of growth, potash manures containing chlorides have not on the average produced any significant improvement in the rate of starch production, but manuring with potassium sulphate has produced a marked improvement. This superiority of the plants treated with potassium sulphate is associated with a more rapid translocation of starch from the leaflets, but does not appear to be wholly due to that factor. In addition to a certain average difference in rate of starch production between differently treated plots there appears to be a significant difference in response to environmental conditions and to age. As between the potassium sulphate treatment and the 'no potash' treatment part of this differential response may be due to a closer association, on the

potash-starved plot, between rate of starch production and intensity of radiation, and part also to a more rapid deterioration with increasing age on the potassium sulphate plot, which starts initially at the higher level.

The general conclusions do not appear to be appreciably affected by the method of expressing the intensity of colour developed in the iodine test for the starch in the leaflets. It seems probable, however, that the units of the colour scale actually used for grading the leaflets (Colour Scale Ridgway 59^{IIII}) do form a scale which gives an approximate measure of changes in starch production content, provided an intense violet black colour is not reached. It is hoped in further work to make a comparison between the iodine test and chemical determinations of the starch content of leaflets. Meanwhile, the elements of order which even these preliminary observations have shown upon analysis justify the belief that the Sachs test may prove of great service for field studies in crop physiology.

SUMMARY.

1. Field observations have been made upon the rate of starch production in leaflets of potato plants grown without potash manure and also with three different types of potash manures, Sachs's iodine test being employed. The observations relate to the later period of growth of the plants.

2. The use of a standard scale of colour tones for grading the colour intensities developed in the leaf by the iodine test is discussed. Two alternative methods for giving numerical values to these colour intensities are put forward: (a) the use of the actual units of the tone scale; (b) the use of a scale of black derived from the colour-wheel analysis of the tone scale.

An analysis of the experimental data for starch production, using either of these methods, leads to the same general conclusions, but evidence is presented which suggests that the units of the particular standard tone scale employed form the better approximation to a measure of starch content. The tone scale found to be satisfactory is tone scale 59^{IIII} of Ridgway's 'Colour Standard and Nomenclature'.

3. The observed variation in starch production is analysed statistically, and it is shown that while the application of potash manures containing chlorides has not appreciably improved the rate of starch production, the application of potassium sulphate has done so. At the same time also the rate of translocation of starch from the leaflets on the potassium sulphate plot is increased.

The writer wishes to record his special indebtedness to Mr. Catley, of the University of Sydney, and Mr. Dawson, of the University of Manchester, who began the work with him, and were almost entirely responsible for the series of observations made. Thanks are also due to Mr. Eden for assistance with the sampling and in other ways.

The Effect of Ionized Air on the Rate of Respiration of Barley Seedlings.

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With eight Figures in the Text.

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I. INTRODUCTION.

THE question of the effect of ionization of the air on the physiological processes of the plant is one which has received very little attention. Air is always ionized to a slight extent, but it is still uncertain as to whether this is of any physiological importance to the plant, or whether greater degrees of ionization cause any change in plant processes.

The results recently obtained by Blackman and his co-workers (1, 2, 3, and 5) on the growth of plants exposed to a high-tension electric discharge suggested the importance of further work on this subject, for with the high voltages used in such discharges the ionization of the air is much increased. All or part of the effect of the discharge might be due to the high ionization of the air, rather than to the current passing through the plant.

H. A. Spoehr (7) in 1915 found that the rate of respiration of various plants, notably wheat and onions, was lower at night than during the day.

He suggested that this difference in rate might be due to periodic differences in the degree of ionization of atmospheric air. In a series of experiments, in which the air supplied to germinating wheat seedlings was artificially de-ionized, the day-rate only exceeded the night-rate by 1.4 per cent., whereas in normal air the excess was 9.1 per cent. For another set of wheat seedlings the relative figures were 1 per cent. for de-ionized air, and 4.2 per cent. for normal air. Beyond a general record of the climatic conditions during the experiments, there is no indication as to the degree of ionization of the air to which the plants were subjected.

Stoppel (8) in 1920 claimed a definite increase in the rate of respiration of cut shoots when the ionization of the air supplied to them had been increased by passing through a tube containing the radio-active mineral, carnotite. The change in the ionization of the air was shown by an increase of electrical conductivity of from two to six times the normal. Stoppel, using the baryta method, carried out a number of experiments, but with two exceptions numerical data are not given; there is merely the statement that an increase was observed as a result of exposing the shoots to air passed through the tube holding the carnotite. In the case of the two series for which data are given, the increase in the rate of respiration was very small, varying from 0.3 per cent. to 1.13 per cent. No results are available as to the regularity of respiration of the control plants during successive periods, and there is no basis for calculating the probable errors, which must almost certainly have been as large or larger than the increases observed. The results, therefore, cannot be considered in any way conclusive.

The need of further work in this field was thus very obvious, and the following investigation was accordingly undertaken with a view to determining the effect of varying degrees of ionization on the rate of respiration of green plants.

II. METHOD OF EXPERIMENTATION.

The method employed was to determine the respiration of barley seedlings over a period of seven hours, taking hourly readings of the carbon dioxide production. At specified times the plants were subjected to ionized air, and the resulting rate of respiration was compared with that of control plants exposed to normal air.

(a) *Germination of Seeds.*

A 'pure line' of barley (var. *Goldthorpe*), obtained from the cereal station of the Department of Agriculture and Technical Instruction for Ireland, was employed. A number of seeds of approximately uniform size and colour were selected each day, placed between filter-papers in a Petri dish, and moistened with 8-10 c.c. of tap-water. After twelve to fifteen hours the germinating

seeds were transferred to a large crystallizing dish, and planted out on glass-wool and sterilized glass beads soaked in 50 c.c. of culture solution.¹ On the seventh day the seedlings were used for experiment, their height being about 8–11 cm. The temperature during germination varied between 21° C. and 22.5° C.

For the last set of experiments carried out in December, January, and February, 1925–6, the germinating seedlings were supplied with additional light from a gas-filled Osram lamp (100 watts) at a distance of 18–20 cm. above the seedlings. This artificial illumination was supplied from 10 a.m. to 5 p.m. daily.

(b) Apparatus.

To measure the rate of respiration, the seedlings were placed in a glass chamber of volume 6.5 litres, with a flat glass lid, made airtight by smearing well with vaseline. The lid was bored with three holes to carry the corks bearing (1) the air inlet tube, (2) the outlet tube, and (3) the rod to which the polonium was attached. The respiratory chamber was placed in the dark inside an electric incubator, regulated to a temperature of 22° C. A continuous current of air was drawn through at the rate of 90–110 litres per hour, by means of a water-pump, the pressure being regulated by means of a mercury manometer. The atmospheric air supplied to the seedlings was drawn from outside the laboratory, and was passed through three soda-lime tubes and then over sticks of caustic soda, in order to ensure the removal of all traces of carbon dioxide. The absorption of the product of respiration was carried out by bubbling the air current through two Reiset towers, each containing 75 c.c. of standardized N/5 NaOH. In earlier experiments a baryta 'trap' was introduced into the system between these towers and the pump in order to detect any indication of incomplete absorption. The result, however, was so uniformly negative that this precaution was considered unnecessary in the later experiments. The use of the Reiset tower has a great advantage over that of the Pettenkofer tube in that absorption is complete even with a rapid air-stream. It was thus possible to change the volume of air passing over the plants once in four minutes, so that there was no risk of the carbon dioxide accumulating in the respiratory chamber.

At the end of each hour the stopcocks on each side of the two Reiset towers were closed, so that the air-flow through the system ceased. The towers were then removed, and washed down with distilled water. The carbon

¹ The culture solution used was made up as follows :

KNO ₃	1 gm.	CaSO ₄	0.5 gm.	} Dissolved in 1,000 c.c. distilled water. Diluted to one-tenth strength when used.
KH ₂ PO ₄	0.25 gm.	MgSO ₄	0.5 gm.	
FeCl ₃	2 c.c. 5 per cent. solution			

dioxide was estimated by means of the double titration method, the carbonate in the solution being converted into bicarbonate with $N/2$ HCl, and the total bicarbonate present then titrated with $N/20$ HCl. The 'Universal Indicator' (British Drug Houses, Ltd.) recommended by Plimmer (4) was found to be very convenient, the end points being respectively yellow-green and red.

The first reading was taken at $1\frac{1}{2}$ to $1\frac{3}{4}$ hours after the vessel holding the plants had been placed in the incubator. This reading gave a mean with a relatively high probable error, which indicated that the plants took time to recover from the effect of transference from the germinator to the incubator. The second, third, and fourth hour readings gave approximately uniform results, and there was a tendency to a decrease in rate during the fifth and sixth periods. It was therefore decided that the second, third, and fourth hours should be used for purposes of comparison. The second hour reading was taken as the standard rate; the ionizing agent was introduced during the third or the fourth period, removed for one hour, and introduced again during the fifth or sixth hour. Any change from the standard rate of the second hour which was associated with the introduction of the ionizing agent was then compared with any slight change from this rate exhibited by the control plants during the hours in question.

The results are expressed as percentage increases or decreases from the standard rate of the second hour.

The ionizing agent which was employed was polonium (radium F), deposited on a copper foil and obtained from the late F. H. Glew. It emits only α particles of low penetrating power, with a range of 3.8 cm. in air, and the period of activity is short, decreasing in strength at the rate of about 0.5 per cent. per day. This piece of foil (about 1 cm. by 3 cm.) was attached to the end of a glass rod by means of sealing-wax.

In the experimental sets referred to as *B*, *D*, and *E*, the central cork in the lid of the respiratory chamber was replaced by a cork bearing this rod to which the polonium was sealed. In set *A* the polonium was also inside the vessel, but it was placed at one side so that the polonium surface was vertical, and at a distance of 4–5 cm. from the seedlings. In set *C* a difficult method was adopted. A glass bulb of 4 cm. diameter was blown on the air inlet tube, but outside the respiratory vessel, and the copper foil bearing the polonium was sealed inside this bulb. In this way the air was ionized before it entered the chamber containing the plants.

The following diagrams (Figs. 1–3) will explain the arrangement in the different experiments.

Sets *A* and *B* with a corresponding control were carried out between October and December, 1924; set *C* in January and February, 1925; and sets *D* and *E*, each with a control set, were completed by July, 1925, and February, 1926, respectively. An additional series with controls was also

carried through in October and November, 1925. Although the results of the latter pointed to the same conclusion as that drawn from sets *A*, *B*, *D*, and *E*, the light intensity during germination of the seedlings was very

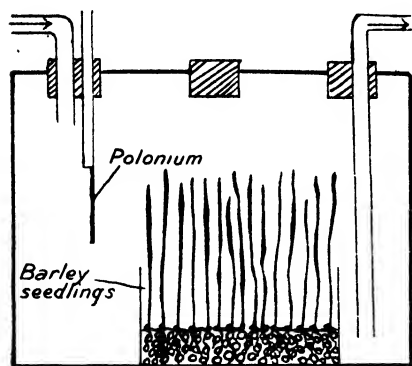


FIG. 1. Diagram showing the relative positions of the seedlings and the polonium in experimental set *A*.

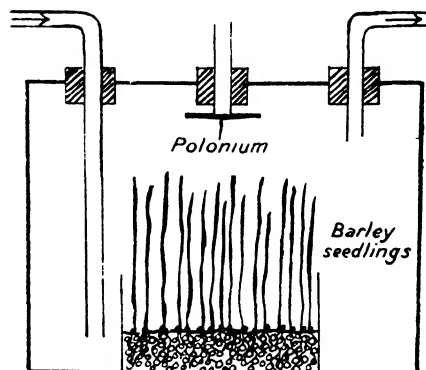


FIG. 2. Diagram showing the relative positions of the seedlings and the polonium in experimental sets *B*, *D*, and *E*.

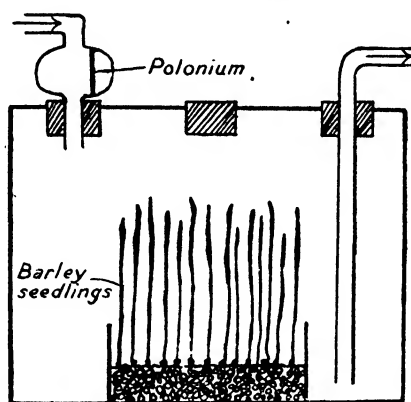


FIG. 3. Diagram showing the relative positions of the seedlings and the polonium in set *C*.

variable, and the growth of the plants irregular; hence these data are not included in the results given in Table III.

A series of blank experiments were carried out at frequent intervals, and tests for the occurrence of a leak were made daily.

(c) Determination of the Degree of Ionization.

The degree of ionization was estimated by measuring the saturation current between a charged and an 'earthed' copper disc, diameter 10–8 cm. These were placed 5 to 2 cm. apart in the respiratory chamber in the position normally occupied by the seedlings. The wires holding the discs

were well insulated by quartz tubing passing through rubber corks. One disc was charged by means of dry batteries giving a range of 1,068 volts. The second disc was earthed through a highly sensitive galvanometer of the moving coil type, with a resistance of 2,180 ohms and giving a deflexion of 20,000 mm. per micro-ampere. A less sensitive instrument which gave a deflexion of 1,200 mm. per micro-ampere was used for the determination of the ionization in sets *D* and *E*. Determinations were made both in still air and with the current of air moving at the usual rate of 90–110 litres per hour; the results were, however, the same in both cases.

Table I gives the results of the determinations. The ionization is expressed in the form

$$\frac{\text{No. of ions per c.c.}}{\text{No. of ions per c.c. in normal air}}$$

The number of ions per c.c. in normal atmospheric air is taken as 500 positive and 500 negative, as given by Wilson (9). The different degrees of ionization were obtained by varying the position of the polonium, and by using polonium of different ages, i. e. at different stages of disintegration.

TABLE I.

Ionization Determinations.

The positions of the polonium were as shown in Figs. 1, 2, and 3, the seedlings being replaced by the two copper discs. In *A*, *B*, *D*, and *E* the polonium was inside the respiratory vessel; placed centrally above the discs in *B*, *D*, and *E*, and at one side in *A*. In set *C* the polonium was outside the chamber. Normal air is assumed to contain 500 positive and 500 negative ions per c.c.

<i>Experimental Set.</i>	<i>Distance between Discs (cm.).</i>	<i>Volume of Air between Discs (c.c.).</i>	<i>Voltage for Saturation Current (volts).</i>	<i>Deflexion (mm.).</i>	<i>Saturation Current (amp.).</i>	<i>No. of Ions per c.c.</i>	<i>Degree of Ionization. (Normal Air = 1.)</i>
<i>A</i> (see Fig. 1)	3.5	309	652	25	1.25×10^{-9}	2.5×10^7	2.6×10^4
<i>B</i> (see Fig. 2)	5	441	600–623	30.5	1.53×10^{-9}	2.2×10^7	2.2×10^4
<i>C</i> (see Fig. 3)	3.5	309	947	very small	—	—	1 (approx.)
<i>D</i> (see Fig. 2)	3	151	947	36	3.0×10^{-8}	1.6×10^9	1.6×10^6
<i>E</i> (see Fig. 2)	2	100	947	2	1.7×10^{-9}	1.1×10^8	1.1×10^5

III. EXPERIMENTAL RESULTS.

The mean rates of respiration which are given in the following tables and graphs are in all cases based on either ten or twenty experimental results. In determining the value of the results, probable errors have been

calculated in the usual way, a difference that is three times the probable error being taken as significant in either direction.

To give some indication of the absolute amounts of carbon dioxide estimated, and the variations in rate from day to day, the full data of experiments in set *B* are set forth in Table II.

TABLE II.

Set B. Hourly Rate of Respiration of 50 Barley Seedlings, expressed in milligrams of carbon dioxide.

November—December, 1924.

Date.	1st Hour.	2nd Hour.	3rd Hour	4th Hour.	5th Hour.	6th Hour.
Nov.				Polonium present.		Polonium present.
14th	4.16	4.81	5.70	6.69	4.16	6.04
21st	3.52	4.62	4.80	4.80	3.88	4.06
25th	4.57	4.28	4.79	4.99	4.12	6.60
26th	3.00	3.26	3.90	3.26	3.60	4.81
27th	2.83	4.08	4.17	4.40	5.24	3.98
28th	3.21	3.69	3.57	3.78	3.13	4.41
Dec.						
1st	4.66	4.91	5.09	6.19	5.43	5.59
3rd	3.67	3.91	4.08	4.15	3.26	4.08
4th	3.90	4.12	3.90	5.02	4.02	5.13
5th	5.11	4.91	4.91	5.50	4.82	4.43
Mean Rates	3.95	4.26	4.49	4.88	4.17	4.91

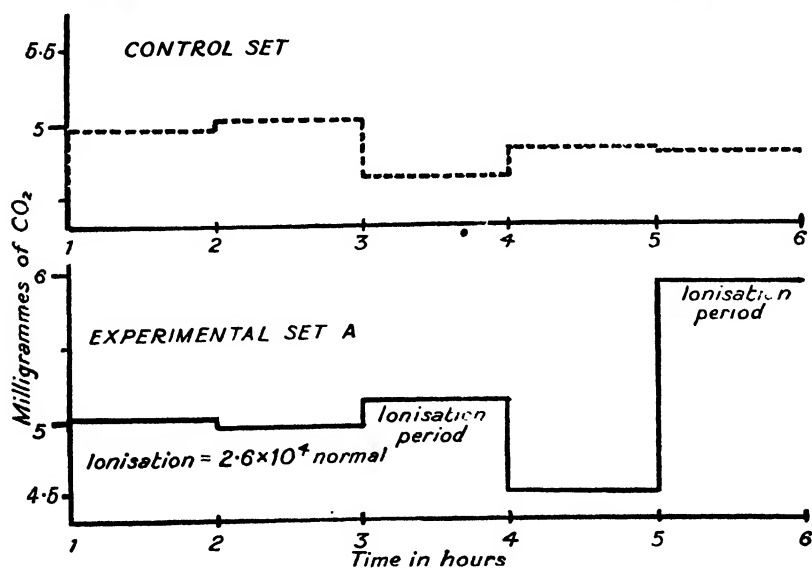


FIG. 4. Graph showing mean rates of respiration during the six hourly periods of the control and the experimental sets of *A*.
Ionization rate = 2.6×10^4 normal.

Figs. 4 to 7 show graphically the mean rates calculated for each experimental set, together with a corresponding control series, except in the case of set *C*, for which no controls were carried out.

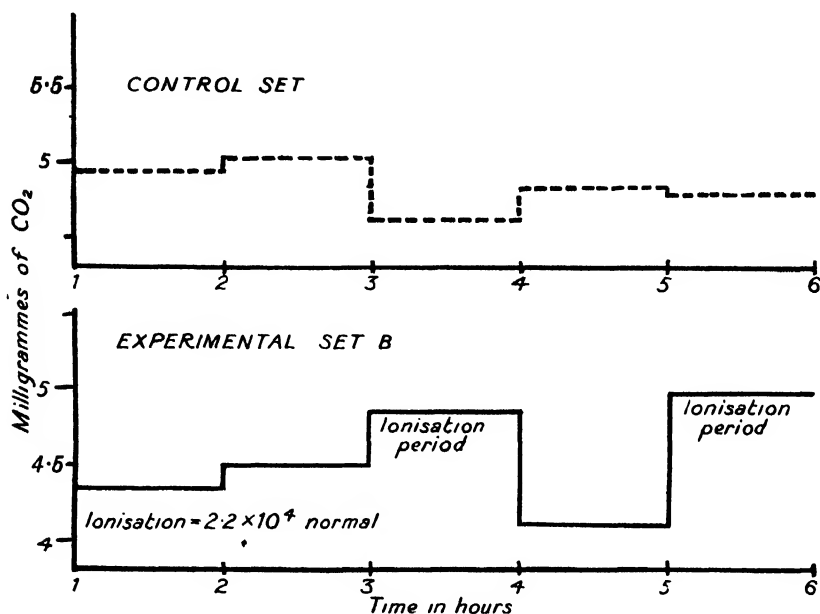


FIG. 5. Graph showing mean rates of respiration during the six hourly periods of the control and the experimental sets of *B*.
Ionization rate = 2.2×10^4 normal.

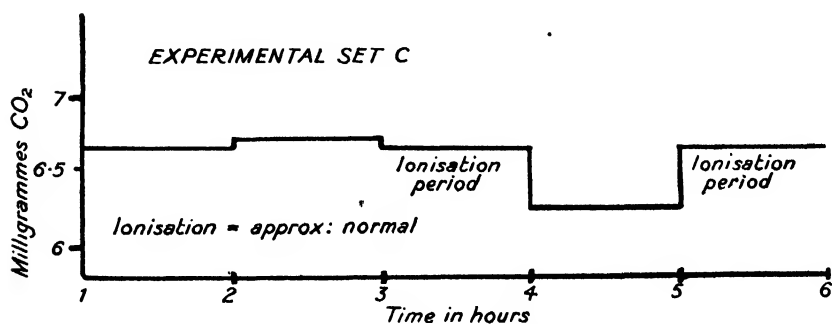


FIG. 6. Graph showing mean rates of respiration during the six hourly periods of the experimental set *C*.
Ionization = no appreciable increase over normal.

It will be observed that in experimental series *A*, *B*, *D*, and *E*, the application of the polonium produces in each instance an increase in the rate of respiration.

All the experimental results are incorporated in Table III.

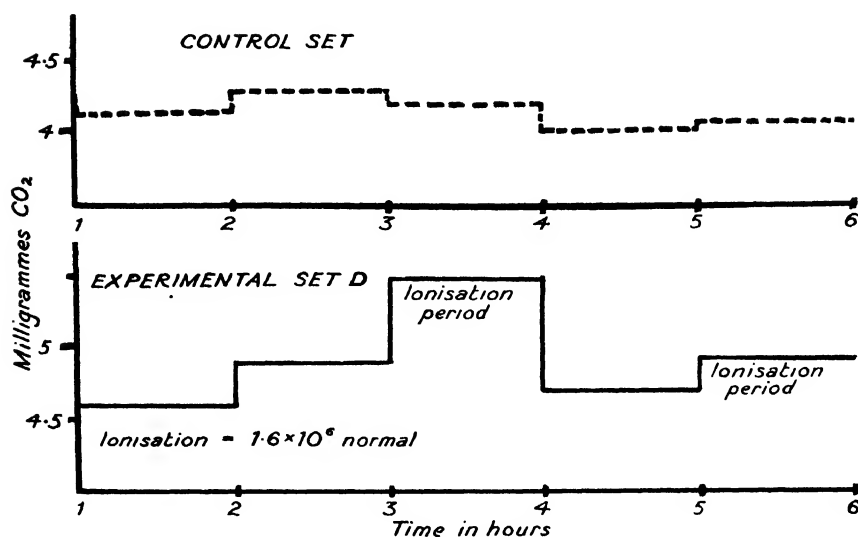


FIG. 7. Graph showing mean rates of respiration during the six hourly periods of the control and the experimental sets of *D*.
Ionization rate = 1.6×10^6 normal.

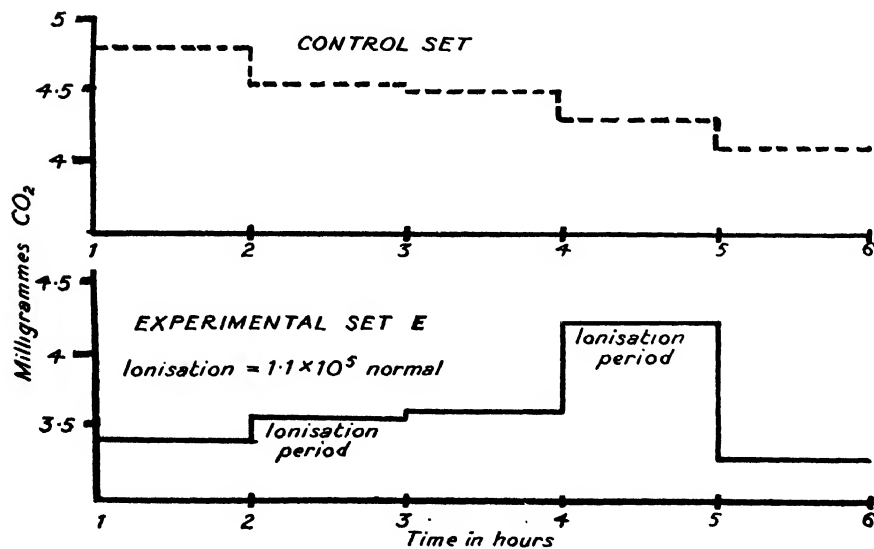


FIG. 8. Graph showing mean rates of respiration during the six hourly periods of the control and the experimental sets of *E*.
Ionization rate = 1.1×10^5 normal.

TABLE III.

The position of the polonium in the various sets is shown in Figs. 1, 2, and 3. The rate of respiration observed is expressed as a percentage increase or decrease over the rate of the standard second hour.

<i>Experimental Set.</i>	<i>Hour of Experiment.</i>	<i>Percentage Difference from Rate of Respiration of Standard 2nd Hour.</i>		<i>Degree of Ionization. (Normal Air = 1.)</i>	<i>Percentage Increase of Respiration due to Ionization.</i>
		<i>Controls.</i>	<i>Ionization.</i>		
<i>A</i> (polonium placed on one side of seedlings)	4th	-6.21 ± 3.34	$+ 4.8 \pm 6.18$	2.6×10^4	11.03 ± 7.03
	6th	-2.89 ± 2.81	$+ 18.81 \pm 5.37$		21.70 ± 6.06
<i>B</i> (polonium placed centrally and above seedlings)	4th	-6.21 ± 3.34	$+ 13.08 \pm 2.61$	2.2×10^4	19.29 ± 4.24
	6th	-2.89 ± 2.81	$+ 16.62 \pm 4.78$		19.50 ± 4.45
<i>C</i> (polonium outside respiratory chamber)	4th		$+ 1.27 \pm 2.26$	1 (approx.)	
	6th		$+ 0.97 \pm 2.56$		
<i>D</i> (polonium placed centrally and above seedlings)	4th	$+ 2.90 \pm 3.77$	$+ 20.42 \pm 5.01$	1.6×10^6	13.52 ± 6.27
	6th	-2.70 ± 2.71	$+ 6.81 \pm 5.59$		9.51 ± 6.71
<i>E</i> (polonium placed centrally and above seedlings)	3rd	-1.11 ± 1.58	$+ 4.47 \pm 2.03$	1.1×10^5	5.58 ± 2.57
	5th	-3.88 ± 2.81	$+ 25.23 \pm 4.86$		29.11 ± 5.62

IV. DISCUSSION.

Table III shows that *in all cases* higher rates of respiration are to be observed when air artificially ionized by polonium is drawn over the barley seedlings, although these increases are not in every instance significant.

In set *C*, as was to be expected, there was no significant effect of the ionizing agent, for the polonium was placed outside the respiratory chamber, and the increased ionization in the chamber was, if any, too slight for determination. The failure to observe any increase of ionization or physiological effect in this set is to be explained by the rapid recombination of the ions.¹ It indicates that the physiological effect, when produced, is due to the ions themselves, and not to any gaseous products (ozone, &c.) associated with such ionization.

In set *B*, where the polonium was directly above the seedlings, and the degree of ionization was low, an increase of significant value was obtained for both periods of treatment.

In set *E*, however, with a slightly higher ionization of the air, there is certainly a significant increase in the second period of application; the increase during the first period is, however, not more than twice the probable error.

¹ Chauveau (4) has shown that for a number of ions of the order ten millions per c.c., the time in which they are reduced to half their initial value at normal pressure and in dry air would be about 0.06 second.

In set *D*, which was otherwise similar to *B* and *E*, but with a still higher degree of ionization, the increase is without significance during the second period, and just significant for the first. In *A* the foil bearing the polonium was placed laterally, so that this set is not quite comparable with the others. It shows a barely significant increase during the first period, but one which is definitely so during the second application.

There can thus be no question that artificially raising the ionization of the air can, within certain limits, cause definite acceleration in the rate of respiration. Before an exact relationship between these changes of respiratory rate and the degree of ionization can be established, much further work is required. The results suggest, however, that with the higher degree reached in set *D*, the concentration is such that little or no acceleration can be obtained. A still greater degree of ionization would probably produce a retardation of respiratory rate.

It is not possible to indicate at present the nature of this effect of highly ionized air on the respiration process. It should be noted, however, that even with the highest degree of ionization used the proportion of molecules actually ionized is exceedingly small, being of the order two in ten thousand millions.

V. SUMMARY.

1. When the air passing over barley seedlings is artificially ionized by means of polonium applied for one hour, intermitted for one hour, then applied again for another hour, the rate of respiration is increased during the periods of application of the polonium. The maximum percentage increase observed was 29.11 ± 5.62 during the second period.

2. The acceleration varies according to the degree of ionization. With ionization 20,000 times that of normal air (assumed to be 500^+ and 500^- ions per c.c.), an increase in rate of respiration resulted from both applications of the polonium. Ionization 100,000 times normal air gives a markedly significant increase in the second period only. With a still higher degree, i. e. 1,000,000 times normal air, there is a barely significant or no increase.

3. The nature of this effect on respiration is at present obscure. It is almost certainly due to the action of the ions themselves, and not to the gaseous products (ozone, &c.) associated with such ionization.

This work was undertaken at the suggestion of Professor V. H. Blackman, whom I have to thank for much help and criticism.

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The Effect of Ionized Air on the Assimilation and Respiration of Green Leaves.

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With three Figures in the Text.

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INTRODUCTION.

THE results obtained in this laboratory by Blackman and his co-workers (1, 3, and 4), on the effect of high-tension electric discharges on plants, raise the question of the physiological effect of ionized air on plant processes. It seemed therefore advisable to undertake some experiments on the effect of air, ionized above the normal, on the processes of respiration and assimilation, a field in which there has been little previous work.

Spoehr (14) and Stoppel (15) have made some observations on the effect of ionized air on respiration; their results are reviewed by Middleton (10) in an accompanying paper in this Journal.

In regard to the effect on assimilation Henrici (7), in 1919, in the

course of her investigations on the assimilation of alpine and lowland plants, states that before a thunderstorm the assimilation of alpine plants is much more active in spite of the poorness of the light.

In an attempt to explain this phenomenon she carried out in 1921 (8) a number of experiments on the influence of the conductivity of the atmosphere on photosynthesis at Basle. In the first series she compared the effect of air, artificially ionized by means of oxide of thorium, on alpine and lowland plants. She found that, for the latter, ionization of the air affects assimilation favourably in weak light, in medium light it has no effect, and in strong light the action retards assimilation. In alpine plants ionization of the air causes an increase in assimilation in weak and medium light, but in strong light neither increases nor decreases assimilation. Two further series of experiments were carried out, but in these only alpine plants were employed, and a comparison made of the effect on them of normal and of de-ionized air. The results of the experiments carried out at Muottas Muraigl (2,456 m.) show that in weak light ionization of the air causes a large increase in assimilation, while in direct sunlight the rate of assimilation in de-ionized air was nearly as great as that in ionized air. The figures for *Veronica bellidioides* in weak light are 21.7 mg. CO₂ in ionized air, and 0.0 mg. in de-ionized air, and for *Achillea nana* 53.1 mg. CO₂ in ionized air, and 50.3 mg. CO₂ in de-ionized air in direct sunlight. The last set of experiments carried out at Basle, employing electric light, show an increase in assimilation due to ionized air in weak and moderately strong lights, while in very strong light the rate of assimilation in ionized air is considerably less than that in de-ionized air. From her results she claims that in suitable conditions of light ionized air increases photosynthetic activity. In some cases the ratio of the rate of assimilation in ionized air to the rate in de-ionized air was as great as 5-4:1. Her results are not based on the means of a number of experiments from which the probable errors can be calculated, so that it is impossible to estimate their value.

There was clearly need for further work in this field; the investigation reported here was accordingly undertaken.

II. EXPERIMENTAL TECHNIQUE.

Material and Apparatus.

Material. Much time was spent in finding the most suitable leaf for experimental purposes. Experiments were carried out with the leaves of *Abutilon*, *Hydrangea*, *Polygonum*, and *Pelargonium*, of which the last-named proved most satisfactory. Accordingly, throughout the series of assimilation and respiration experiments, the leaves of *Pelargonium zonale* (var. *Paul Crampel*) were employed. In every case leaves were cut and placed in water twenty-four hours before being used, and as far as possible those of approximately the same size were chosen.

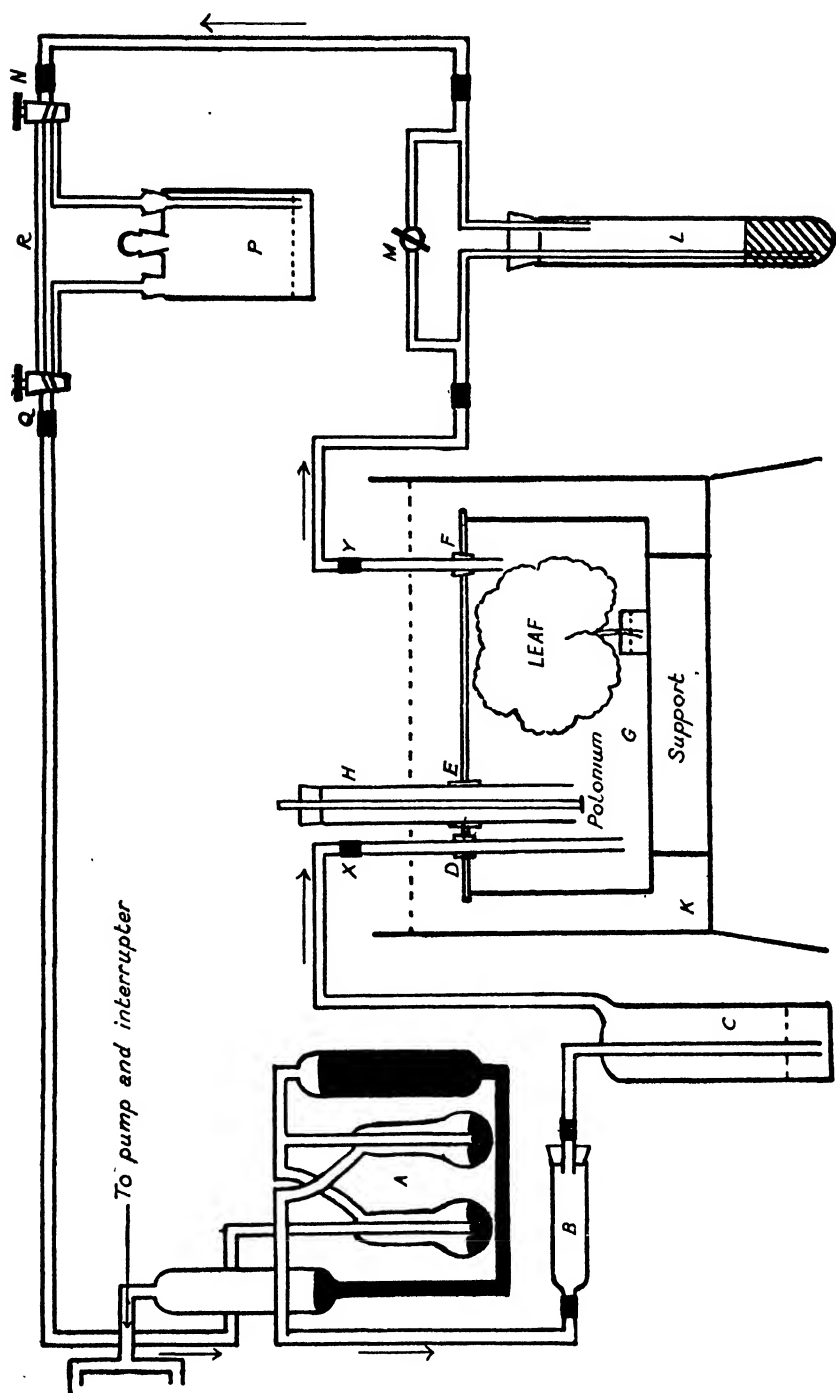


FIG. 1. Diagram (not to scale) of apparatus. For explanation see text.

Method. It was decided to employ for the assimilation work the indicator method elaborated by Bolas (5) in this laboratory. The indicator used was the sodium salt of brom-cresol-purple buffered with sodium carbonate, in aqueous solution, and the colour of the indicator was matched against coloured glass slides (made by the Tintometer Co., Ltd., Salisbury) as described by Bolas. For this matching the light of a 100-watt gas-filled Osram lamp passed through a specially prepared blue (daylight) glass and was then reflected from a piece of opal glass placed obliquely.

Apparatus. The apparatus was designed for the estimation of assimilation and respiration by circulation of air in a closed system. It is shown diagrammatically in Fig. 1.

A is a mercury 'one-way' valve connected with a water suction-pump and a mercury 'interrupter', as described by Blackman and Bolas (2). From A an air current flowing at the rate, approximately, of 400 c.c. per minute passed through B, a glass tube packed with gold leaf to absorb any mercury vapour from the valve A and containing a dust filter; then it passed through C, a gas wash-bottle containing about 10 c.c. of distilled water, finally entering the leaf-chamber G. The leaf-chamber is a glass vessel ($20 \times 5 \times 10$ c.c.) with a 'ground' lid made air-tight with vaseline, and further secured by two metal clamps; it is sunk in a water-bath, K, electrically controlled and kept at 25°C . The lid of G is perforated in three places, D, E, and F, to admit three corks carrying (1) the inlet tube, (2) the tube for bearing the ionizing material, and (3) the outlet tube. The leaf is placed in the chamber with its stalk immersed in water in a small glass cup; it is kept in a vertical position, and supported at the back by a piece of bent glass tubing attached to the end of the outlet tube. The air after passing over the leaf emerges at F and may then pass through the indicator contained in the Pyrex glass tube L, or, if the stopcock M is open, straight through M, N, R, and Q, and so back to A. N and Q are 'two-way' stopcocks which enable the gas stream to be diverted from R through the Woulff's bottle P. Carbon dioxide can be supplied to the system by placing in P 50 c.c. of soda-water (prepared in a 'sparklet' syphon), and circulating the air of the system through it for an appropriate time.

The apparatus employed for matching the colour of the indicator with that of the glass slips is shown in Fig. 2, and is of the same type as that described by Bolas (5).

The small water-bath shown in the figure is kept at a temperature of 25°C . by a steady flow of warm water. It holds the two similar Pyrex tubes, one containing the indicator, and the other distilled water.

Ionization. Polonium provides a very convenient method of ionization, for it gives off only α particles (helium) having a range in air of 3.72 cm. (Roth and Scheel (12)), without the complication of β and γ rays; the α particles have a high ionizing power. The polonium, deposited on a strip of

sheet copper, was obtained from the late Mr. F. Harrison Glew. The copper foil (1 cm.) is fixed to the end of a glass rod with sealing-wax; when the leaf is to be subjected to the action of ionized air a cork, bearing this rod, is inserted into the tube H, which passes through the lid of the leaf-chamber

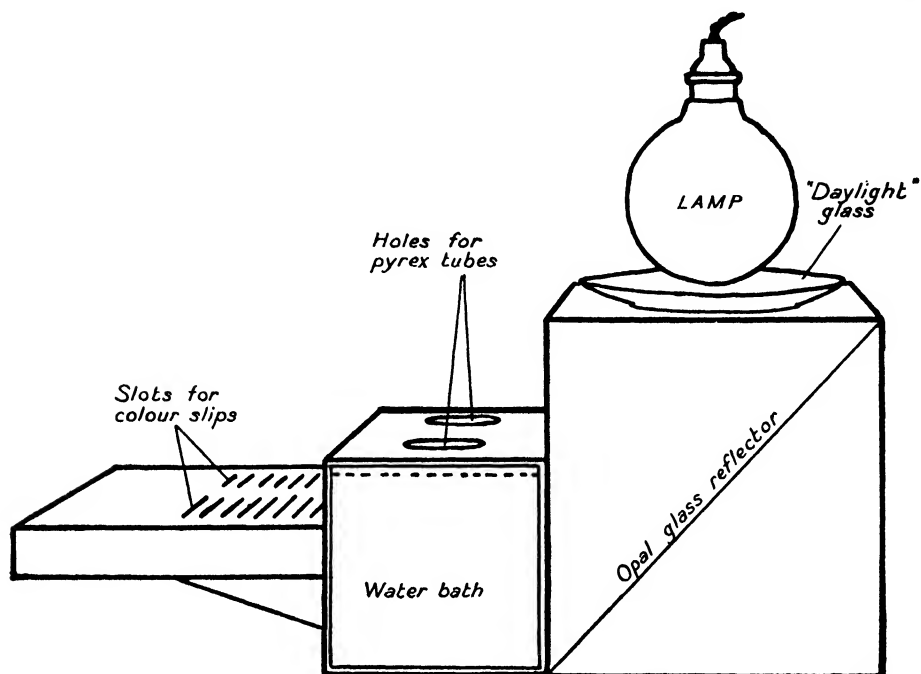


FIG. 2. Diagram (not to scale) of apparatus used for colour matching. For explanation see text.

and is open below. This tube is closed above with a plain cork when the polonium is employed. The copper foil, which is horizontal with its polonium-bearing surface facing downwards, projects just beyond the lower end of the tube. Its distance from the bottom of the vessel and from the nearer edge of the leaf is about 4 cm.

All joints of the apparatus were well vaselined, and rubber tubing was avoided except when absolutely necessary. In such cases thick-walled pressure tubing was used, and care taken that the ends of the glass tubes within it were in contact. From time to time 'blank' experiments were run to test for any leakage of atmospheric air into, or carbon dioxide out of the system.

Determination of Degree of Ionization.

To determine the degree of ionization the saturation current between a charged and an 'earthed plate' was measured. Two copper discs, 8 cm. in diameter and 3.5 cm. apart, were placed in the leaf-chamber, the earthed

plate in the position normally occupied by the leaf. The wires leading from the plates were insulated by means of quartz tubing passing through the lid of the leaf-chamber. The one plate was charged from dry batteries; an e.m.f. of 216 volts was found sufficient for the saturation current, which was measured by means of a Dolezalek electrometer.

Using this method two measurements of the saturation current were made, the most accurate being that of November, 1926, in which a saturation current of 5×10^{-12} amp. was found giving the number of ions as 1.82×10^5 per c.c. The results of apparent assimilation and respiration given in this paper were obtained between February and June, 1926. As the half-life period for polonium is 136 days (13) one can calculate the degree of ionization for the beginning and end of the experiments. Following Wilson (16) in taking normal air as containing 1,000 ions per c.c. (500^+ and 500^-), the degree of ionization, compared with air, was at the beginning of the experiments 7.28×10^2 and at the end 3.64×10^2 .

There was one small difference between the conditions when the leaf was in the chamber and when the ionization measurements were made. In damp air, such as that to which the leaf was exposed, it was found impossible to measure the saturation current as the leaks were too great; ionization measurements were accordingly made in air dried with calcium chloride. It is interesting to note that no difference was to be observed between measurements made in still air and in air circulating at the normal rate.

Procedure for determining Assimilation Rates.

The leaf was placed in the leaf-chamber, the lid attached, and the whole lowered into the water of the constant temperature bath. The inlet and outlet tubes were connected with the rest of the system at X and Y (Fig. 1) by means of pressure tubing; the pump was then set in action. The upper surface of the leaf was illuminated by a 100-watt gas-filled Osram lamp placed vertically in such a position that the leaf was 15 cm. from the centre of the ring-shaped horizontal filaments.

The indicator was found to work most satisfactorily between a purplish grey, given by the combination of 3.25 Blue, 2.5 Red, and 0.25 Yellow slides (set I), and a purple given by the combination of 4.0 Blue, 3.5 Red, 0.0 Yellow slides (set II). A colour change of this range indicates a decrease in the concentration of carbon dioxide from about thirty-five parts to twenty-one parts in 10,000. The volume of the system being 1,350 c.c., such a change denotes an assimilation of 1.95 c.c. CO_2 or 3.91 mg. CO_2 . Higher concentrations of CO_2 could have been used, but it was thought advisable to keep the maximum concentration at a level of about ten times the normal.

A fresh preparation of indicator was put in the indicator tube L (Fig. 1)

and set I of the colour slips (i. e. 3.25 Blue, 2.5 Red, 0.25 Yellow) placed in position. The air current was made to pass through P for about 10 seconds by turning the stopcocks Q and N; the current was then directed through R. After three and a half minutes (the time taken for the whole volume of air to pass round the system), during which the stopcock M was closed so that the air bubbled through the indicator, a comparison of the latter and the slides was made. Usually it was observed that the indicator appeared slightly more yellow in colour than the slips, showing that the carbon dioxide concentration was too high. If the difference in colour was marked the tap of the stopcock Q was removed so that ordinary air entered the system and reduced the concentration of carbon dioxide. If the difference was slight the leaf was allowed to assimilate until the indicator exactly matched the slips. When satisfactory matching was achieved the time was noted, and the stopcock M opened so that the gas no longer bubbled through the indicator. At intervals the current was directed through the indicator so that the rate of colour-change could be observed. When the colour of the indicator began to approach that of the lower standard, set II of the colour slips was placed in the comparator, and the time determined at which exact matching occurred. When the observation was complete, carbon dioxide was again added to the system and the procedure repeated three more times, so that a set of four readings of assimilation rates for equal amounts of carbon dioxide were available.

In those experiments in which the effect of ionized air was to be tested, the first *two* readings were taken exactly as described above, but before the third period of assimilation the 'blank' cork in the tube for polonium was replaced by the cork bearing the glass rod with the polonium attached. This was withdrawn at the end of the third period. The error due to loss of carbon dioxide when exchanging the corks was found by experiment to be negligible. After the end of the fourth assimilation period the leaf was removed from the leaf-chamber, its surface area estimated by a planimeter, and its 'wet weight' and 'dry weight' determined. The area stated is that of one surface only.

Procedure for determining Respiration Rates.

In determining the rate of respiration the same apparatus was employed with the lamp removed, and the leaf-chamber kept in darkness. The temperature of the chamber and the rate of flow of air remained the same, but the glass-stoppered Woulff's bottle contained 50 c.c. of 0.2 N NaOH (approx.) to absorb the carbon dioxide formed in respiration. The respiratory period employed was two hours, and three readings were always taken in succession during a period of six hours.

In every case before the respiratory rate of the leaf was determined, it was placed in the leaf-chamber with the usual concentration of carbon

dioxide, and its apparent assimilating rate estimated in the way already described. This had two advantages: (1) that of determining whether the assimilation was normal; (2) of ensuring a fair amount of assimilation material for the subsequent period during which respiration was determined. In order that the leaves should be respiring in the dark in concentrations of CO_2 similar to those in the assimilation tests, the first respiration period was begun immediately after completion of the assimilation period, i.e. when the indicator matched set II. of the colour slips and the concentration of CO_2 in the system was 21 parts in 10,000. The carbon dioxide of respiration was allowed to accumulate in the system for a period of two hours, the gas flow was then directed through the vessel P (Fig. 1) containing NaOH, and the circulation so continued until the colour of the indicator again *nearly* matched that of the glass slips of set II. The system was then cut off from P and the gas thoroughly mixed by circulation. Further absorption was then brought about by again passing gas through the sodium hydroxide until *complete* matching was obtained. This process took about five minutes, so that the whole respiration period was a hundred and twenty-five minutes. The carbon dioxide absorbed was determined by double titration with HCl, using the 'Universal Indicator' (British Drug Houses, Ltd.). This reagent, recommended by Plimmer (11), was found to be very satisfactory, the two end points being green and red respectively. Three successive respiratory periods, each of a hundred and twenty-five minutes, were employed for each leaf. In the ionization experiments, polonium was inserted during the second period only.

III. EXPERIMENTAL RESULTS.

Data of Apparent Assimilation.

Controls. The method employed for the determination of assimilation proved very satisfactory, as is shown in Table III, where there is close agreement between the assimilation rates of the four successive periods of any individual leaf. The probable error of the mean is, at its maximum, less than 3 per cent.

Two sets of experiments were carried out: a set in which the assimilation rate of controls was determined and a similar experimental set in which the air was artificially ionized.

In Tables I and II two sample assimilatory results are shown, and in Table III (p. 366) the full assimilation data of all the controls is given. The times for the four periods are, however, not actual times, but those calculated for a standard leaf of 100 cm. surface.

As already pointed out the mean times (i.e. the reciprocals of the rates) for four successive periods show very close agreement.

TABLE I.

Indicator freshly prepared. Colour = 6.5 Blue and 6.0 Red.

Leaf of *Pelargonium zonale* cut and placed in water 24 hours before experiment. Placed in leaf-chamber at 11.30 a.m. CO₂ introduced into the system at 11.50 a.m.

		Colour Slips.			
		Blue.	Red.	Yellow.	
1st period,	12.22 p.m.	3.25	2.5	0.25	} 38 min.
	1.0 "	4.0	3.5	0.0	
2nd "	1.40 "	3.25	2.5	0.25	} 39 "
	2.19 "	4.0	3.5	0.0	
3rd "	2.40 "	3.25	2.5	0.25	} 38 "
	3.18 "	4.0	3.5	0.0	
4th "	3.32 "	3.25	2.5	0.35	} 37 "
	4.9 "	4.0	3.5	0.0	

Area of leaf = 75.15 sq. cm.

Wet weight = 2.41 grm.

Dry " = 0.304 grm.

TABLE II.

Indicator freshly prepared. Colour = 6.5 Blue and 6.0 Red.

Leaf of *Pelargonium zonale* cut and placed in water 24 hours before experiment. Placed in leaf-chamber at 10.45 a.m. CO₂ introduced at 11.10 a.m.

		Colour Slips.			
		Blue.	Red.	Yellow.	
1st period,	11.40 a.m.	3.25	2.5	0.25	} 34 min.
	12.14 p.m.	4.0	3.5	0.0	
2nd "	12.52 "	3.25	2.5	0.25	} 33 "
	1.25 "	4.0	3.5	0.0	
3rd "	1.59 "	3.25	2.5	0.25	} 33 "
	2.32 "	4.0	3.5	0.0	
4th "	3.22 "	3.25	2.5	0.25	} 35 "
	3.57 "	4.0	3.5	0.0	

Area of leaf = 76.26 sq. cm.

Wet weight = 2.597 grm.

Dry " = 0.340 "

Using the data of Table III (p. 366) the difference between two successive periods, together with the probable error of that difference, is shown below:

Difference between 1st and 2nd period is 0.72 ± 0.90 .

" " 2nd and 3rd period is 0.03 ± 0.91 .

" " 3rd and 4th period is 0.14 ± 0.81 .

These results are very satisfactory, for any variation from period to period is well within the probable error and therefore quite without significance; the difference between the second and third periods is as low as 1.2 per cent.

The important point of the regularity of the assimilation rates of successive periods has thus been established. Therefore, in estimating the

effect of ionized air it is quite safe to compare in any leaf the rate of assimilation in the presence of polonium with the rate of the previous period in which polonium was not present.

TABLE III.

Apparent Assimilation. The times given in the last four columns are calculated for a standard leaf of 100 cm.² surface (one side only).

Temp.	Area of Leaf. (One Surface.)	Wet Weight.	Dry Weight.	1st Period.	2nd Period.	3rd Period.	4th Period.
	cm. ²	grm.	grm.	min.	min.	min.	min.
1. 25° C.	68.00	2.035	0.199	20.40	20.40	21.08	20.40
2. "	70.49	1.542	0.171	29.64	33.88	30.35	29.64
3. "	74.37	1.891	0.245	26.03	28.26	27.52	29.00
4. "	75.15	2.410	0.304	28.56	29.31	28.56	27.81
5. "	73.56	2.538	0.307	27.21	27.21	26.48	27.96
6. "	87.60	2.862	0.334	25.40	25.40	28.91	28.03
7. "	63.75	1.834	0.235	24.86	25.51	25.51	24.23
8. "	69.62	2.325	0.303	27.15	26.45	28.54	27.85
9. "	85.15	2.833	0.350	26.49	27.26	26.49	25.55
10. "	76.26	2.597	0.340	25.93	25.17	25.17	26.69
Mean =				26.17 ± 0.53	26.89 ± 0.73	26.86 ± 0.55	26.72 ± 0.606

Ionized Air Experiments.

A series of experiments, similar to those described above for the control series, were undertaken, but the air was ionized by means of polonium during the *third* period. The method of experiment has already been described (p. 363). Two samples of experimental results are shown in Tables IV and V and all the results are brought together in Table VI.

TABLE IV.

Indicator freshly prepared. Colour = 6.5 Blue and 6.0 Red.

Leaf of *Pelargonium zonale* cut and placed in water 24 hours before experiment. Placed in leaf-chamber at 11.15 a.m. CO₂ introduced into system at 11.45 a.m.

Polonium introduced during 3rd Period.

		Colour Slips.			
		Blue.	Red.	Yellow.	
1st period	12.20 p.m.	3.25	2.5	0.25	} 29 min.
	12.49 "	4.0	3.5	0.0	
2nd "	1.34 "	3.25	2.5	0.25	} 26 "
	2.0 "	4.0	3.5	0.0	
3rd "	2.59 "	3.25	2.5	0.25	} 28 "
	3.27 "	4.0	3.5	0.0	
4th "	4.12 "	3.25	2.5	0.25	} 28 "
	4.40 "	4.0	3.5	0.0	

Area of leaf = 82.319 sq. cm.

Wet weight = 2.893 grm.

Dry " = 0.363 "

TABLE V.

Indicator freshly prepared. Colour = 6.5 Blue and 6.0 Red.

Leaf of *Pelargonium zonale* cut and placed in water 24 hours before experiment. Placed in leaf-chamber at 11.10 a.m.*Polonium introduced during 3rd Period.*

		Colour Slips.			
		Blue.	Red.	Yellow.	
1st period	12.38 p.m.	3.25	2.5	0.25	} 36 min.
	1.14 "	4.0	3.5	0.0	
2nd "	2.30 "	3.25	2.5	0.25	} 35 "
	3.5 "	4.0	3.5	0.0	
3rd "	3.55 "	3.25	2.5	0.25	} 34 "
	4.29 "	4.0	3.5	0.0	
4th "	4.42 "	3.25	2.5	0.25	} 36 "
	5.18 "	4.0	3.5	0.0	

Area of leaf = 72.645 sq. cm.

Wet weight = 2.666 gm.

Dry " " 0.351 "

TABLE VI.

Apparent Assimilation. The times given in the last four columns are calculated for a standard leaf of 100 cm.² surface (one side only). The data for the period during which polonium was applied are shown in heavy type in the seventh column.

Temp.	Area of leaf. (One Surface.)	Wet Weight.	Dry Weight.	1st Period.	2nd Period.	3rd Period.	4th Period.
	cm. ²	gram.	gram.	min.	min.	min.	min.
1. 25° C.	83.35	2.389	0.293	39.17	36.68	41.68	37.51
2. "	64.51	1.760	0.226	29.03	30.31	29.68	27.10
3. "	76.19	1.447	0.341	23.62	22.10	28.95	22.86
4. "	97.82	2.990	0.436	29.35	34.24	33.26	32.28
5. "	66.00	2.148	0.334	20.46	23.10	23.10	23.76
6. "	82.32	2.893	0.363	23.87	21.41	23.05	23.05
7. "	67.73	2.639	0.360	27.09	25.74	23.71	24.38
8. "	72.65	2.666	0.351	26.15	25.43	24.70	26.15
9. "	77.93	2.600	0.334	28.83	31.18	31.95	30.33
10. "	73.54	2.220	0.301	23.53	25.74	25.74	24.27
Mean =				27.11	27.59	28.58	27.17
				± 1.10	± 1.12	± 1.25	± 0.98

The mean results for the first and second periods are very similar to the mean results shown in all four periods of the control sets, clearly indicating the regularity of assimilation under these experimental conditions. The probable errors, however, in the present set are somewhat higher, reaching about 4 per cent. This is largely due to the rather aberrant behaviour of leaf No. 1, which shows a rate considerably slower than the mean. The assimilation rate during the third period, when the leaf is exposed to ionized air, is slightly lower (as is shown by the longer time required) than the rate

for the previous two periods and for the subsequent period; the decrease in the rate is only 3 per cent. and is certainly not significant. *Thus no effect on the rate of apparent assimilation has been demonstrated.* It must be pointed out, however, that the existence of quite a marked effect is not precluded. A difference of 4.1 min., i. e. of 15 per cent., would be required to reach significance under the conditions of the experiment.¹

There still remains the interesting question of the effect of ionized air on *real assimilation*, for the determination of which it is necessary to take into account not only the carbon dioxide absorbed from external sources but also that produced in respiration. Accordingly, the respiration of the leaves was determined in the manner described.

Data of Respiration.

Controls. As in the case of assimilation, samples of two complete experiments are given (see Tables VII and VIII). In Table IX (p. 369) all the respiration data are brought together and the mean amounts of carbon dioxide for successive periods calculated. A satisfactory and striking agreement between the three means is noticed, the difference between the highest and lowest readings being only 2 per cent. The regularity of the respiration parts under the experimental conditions was thus established. It is therefore evident that it is quite safe to compare the rate of respiration during the period when the leaf is exposed to ionized air with the period immediately preceding, which may thus be taken as the control period.

TABLE VII.

Indicator freshly prepared. Colour = 6.5 Blue and 6.0 Red.

Leaf of *Pelargonium zonale* cut and placed in water 24 hours before experiment. Placed in leaf-chamber at 10.45 a.m. CO₂ introduced into system at 11.10 a.m. HCl = 0.052 N.

	<i>Colour Slips.</i>			
<i>Assim.</i>	<i>Blue.</i>	<i>Red.</i>	<i>Yellow.</i>	
11.45 a.m.	3.25	2.5	0.25	} 46 min.
12.31 p.m.	4.0	3.5	0.0	
<i>Apparatus darkened.</i>				
<i>Resp.</i>				
12.31 p.m. }	4.0	3.5	0.0	0.55 c.c. HCl, 1.27 mg. CO ₂
2.31 " }				
2.31 " }	4.0	3.5	0.0	0.55 c.c. HCl, 1.27 mg. CO ₂
4.31 " }				
4.31 " }	4.0	3.5	0.0	0.6 c.c. HCl, 1.38 mg. CO ₂
6.31 " }				

Area of leaf = 73.29 sq. cm.

¹ This is calculated on the basis of nine experiments with the aberrant leaf No. 1 excluded, and the probable errors accordingly reduced. If this leaf is included in the means, the size of the probable errors are as given in the table, and a somewhat higher difference would be required for significance.

TABLE VIII.

Indicator freshly prepared. Colour = 6.5 Blue and 6.0 Red.

Leaf of *Pelargonium zonale* cut and placed in water 24 hours before experiment. Placed in leaf-chamber at 11 a.m. CO₂ introduced into the system at 11.30 a.m.

	Colour Slips.			
Assim.	Blue.	Red.	Yellow.	
11.45 a.m.	3.25	2.5	0.25	} 29 min.
12.14 p.m.	4.0	3.5	0.0	
Apparatus darkened.				
Resp.				
12.15 p.m. }	4.0	3.5	0.0	0.7 c.c. HCl, 1.61 mg. CO ₂
2.15 „ }				
2.15 „ }	4.0	3.5	0.0	0.8 c.c. HCl, 1.8 mg. CO ₂
4.15 „ }				
4.15 „ }	4.0	3.5	0.0	0.0 c.c. HCl, 1.61 mg. CO ₂
6.15 „ }				
Area of leaf = 80.10 sq. cm.				

Area of leaf = 80.10 sq. cm.

TABLE IX. *Respiration—Controls.*

Temp.	Area of Leaf. (One Surface.)	Assimilation Times.	Respiration in mg. CO ₂ per 100 cm. ² Leaf Surface.		
			1st Period.	2nd Period.	3rd Period.
	cm. ²	min.			
1. 25° C.	72.00	30	1.71	2.05	2.39
2. „	82.50	30	2.36	1.60	1.08
3. „	73.29	46	1.66	1.66	1.80
4. „	86.00	33	2.05	1.80	1.80
5. „	73.93	35	2.39	2.09	1.66
6. „	67.70	40	2.61	2.28	2.61
7. „	76.32	35	1.49	2.03	1.74
8. „	77.73	40	1.84	2.41	2.41
9. „	80.10	29	1.93	2.21	1.93
10. „	73.60	30	1.35	1.56	1.94
Mean =			1.94 ± 0.088	1.98 ± 0.061	1.94 ± 0.095

Experiments with Ionized Air.

Experimental Results. A number of experiments were undertaken in which, after a preliminary period of assimilation as already described, the respiration of leaves was estimated, first during a two-hour period without polonium, then for a similar period with polonium, then finally for a third two-hour period without polonium. Sample experimental results are given in Tables X and XI and the results of all the experiments brought together in Table XII.

Inspection of the mean results of respiration during three two-hour periods (Table XII) shows that the action of polonium is to increase markedly the rate of respiration, the mean rising from 1.75 mg. for the first period to

3.25 mg. during the second period when the air was ionized by means of polonium, i. e. a percentage increase of 85.7 ± 7.1 , the difference being about twelve times the probable error. It should also be noticed that the respiratory rate in the third period after the polonium has been removed does not return to the level of the first or control period. There is definite *after-effect* shown as a significant percentage increase of 28.0 ± 7.3 over that of the control rate. These results are shown graphically in Fig. 3.

TABLE X.

Indicator freshly prepared. Colour = 6.5 Blue and 6.0 Red.

Leaf of *Pelargonium zonale* cut and placed in water 24 hours before experiment. Placed in leaf-chamber at 10.15 a.m. CO_2 introduced into system at 10.40 a.m. $\text{HCl} = 0.061 \text{ N}$.

	Colour Slips.			
Assim.	Blue.	Red.	Yellow.	
10.56 a.m.	3.25	2.5	0.25	} 33 min.
11.29 "	4.0	3.5	0.0	
<i>Apparatus darkened.</i>				
<i>Resp.</i>				
11.30 a.m. }	4.0	3.5	0.0	0.55 c.c. HCl, 1.49 mg. CO ₂
1.30 p.m. }				
1.30 " }	4.0	3.5	0.0	1.2 c.c. HCl, 3.24 mg. CO ₂
3.30 " }				
3.30 " }	4.0	3.5	0.0	0.95 c.c. HCl, 2.57 mg. CO ₂
5.30 " }				

Area of leaf = 85.31 sq. cm.

Wet weight = 2.282 grm.

Dry " = 0.1996 "

Polonium introduced from 1.30 to 3.30.

TABLE XI.

Indicator freshly prepared. Colour = 6.5 Blue and 6.0 Red.

Leaf of *Pelargonium zonale* cut and placed in water 24 hours before experiment. Placed in leaf-chamber at 10.5 a.m. CO_2 introduced into system at 10.30 a.m.

	Colour Slips.			
Assim.	Blue.	Red.	Yellow.	
11.5 a.m.	3.25	2.5	0.25	} 41 min.
11.46 "	4.0	3.5	0.0	
<i>Apparatus darkened.</i>				
<i>Resp.</i>				
11.46 a.m. }	4.0	3.5	0.0	0.55 c.c. HCl, 1.51 mg. CO ₂
1.46 p.m. }				
1.46 " }	4.0	3.5	0.0	1.0 c.c. HCl, 2.74 mg. CO ₂
3.46 " }				
3.46 " }	4.0	3.5	0.0	0.6 c.c. HCl, 1.65 mg. CO ₂
5.46 " }				

Area of leaf = 79.77 sq. cm.

Wet weight = 2.024 grm.

Dry " = 0.194 "

Polonium introduced from 1.46 to 3.46.

TABLE XII.

Respiration. Polonium applied during 2nd Period.

Temp.	Area of Leaf. (One Surface.)	Wet Weight.	Dry Weight.	Assim. Rate.	Respiration in mg. CO ₂ per 100 cm. ² Leaf-Surface.		
					1st Period.	2nd Period.	3rd Period.
	cm. ²	gm.	gm.	min.			
1. 25° C.	76.32	2.20	0.164	32	1.34	2.89	1.87
2. "	85.31	2.28	0.200	33	1.68	3.65	2.89
3. "	75.67	3.08	0.209	34	1.89	3.08	2.74
4. "	72.16	1.86	0.193	33	1.82	2.37	1.46
5. "	85.74	2.52	0.288	31	1.69	3.07	2.30
6. "	76.77	2.02	0.194	41	1.89	3.43	2.06
7. "	61.67	1.75	0.179	37	2.13	3.85	2.57
8. "	78.83	2.02	0.229	34	2.01	3.18	1.84
9. "	78.63	2.23	0.234	35	1.50	3.85	2.84
10. "	81.28	2.18	0.197	35	1.48	3.19	1.75
Mean =					1.75 ±0.053	3.25 ±0.075	2.24 ±0.108

It is clear that this marked increase in the respiratory rate under the action of air ionized by means of polonium makes valueless any comparison between *apparent* assimilation rates with, and without, polonium. The only satisfactory basis of comparison is that of *real assimilation* rates, where the

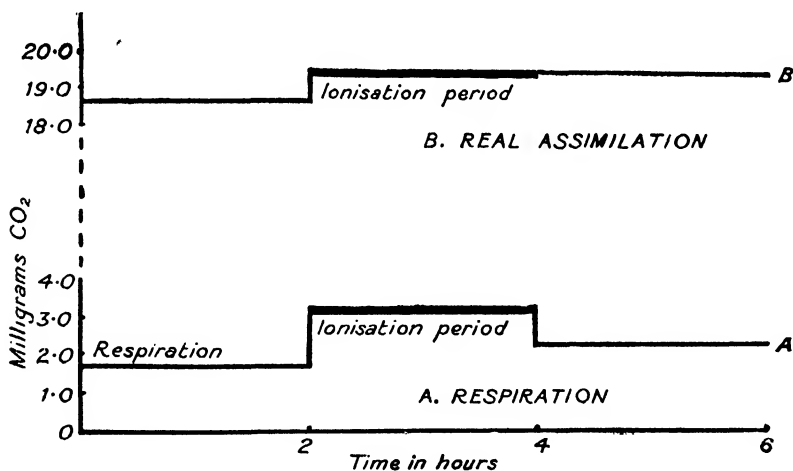


FIG. 3. Graph showing the effect of ionized air on respiration (A) and real assimilation (B). Both real assimilation and respiration are given in terms of mg. CO₂ per two hours; the data for real assimilation are calculated from those for shorter periods given in Table VI (p. 367). The ionization period is shown by a thicker line.

assimilation of carbon dioxide of respiration is taken into account. It is evident that to reach the same rate of apparent assimilation a leaf exposed to air acted on by polonium has to assimilate a much larger amount of *respiratory* carbon dioxide than a control leaf; the experimental leaf might be assimilating at a faster rate than the control, yet its *assimilation time* might be the same or even higher.

Data of Real Assimilation.

Real assimilation rates can be calculated from apparent assimilation rates by taking into account the amount of respiration during any given period of assimilation.

If S equals the real assimilation, A the apparent assimilation, and R the amount of CO_2 produced during time of apparent assimilation, then

$$S = A + R.$$

During the mean control period of 27.59 min., the amount of external carbon dioxide assimilated was 3.91 mg., and 0.41 mg. were respired; the real assimilation during the period is thus 4.31 mg. During the mean experimental period, with ionized air, of 28.58 min., 3.91 mg. together with 0.77 mg. of carbon dioxide of respiration are assimilated, giving a total of 4.68 mg. If S is the real assimilation rate of the control period and S_1 that of the experimental period with ionized air, then

$$\frac{S_1}{S} = \frac{4.68}{28.58} \times \frac{27.59}{4.31} = 1.048.$$

The real assimilation rate in the presence of polonium thus gives a rate about 5 per cent. higher than that of the control, a result shown graphically in Fig. 3. This positive result is too small to be significant, for the probable error is of the order of 6 per cent. It indicates, however, the possibility that, in addition to the very marked increase in respiratory rate, there is also an increase in actual assimilation. The matter can only be decided on the results of a larger number of experiments, by which means the probable error could be considerably reduced.

IV. DISCUSSION OF RESULTS.

In the present state of our knowledge of the nature of the respiratory processes in plants, the mode of action of ionized air cannot be further defined. The effect is probably to be ascribed to some direct physical action of ions on the living cells.

Lind (9) has shown that the ionization of air by means of α rays is associated with chemical changes, e.g. the production of a small amount of ozone, the limiting maximum value for the number of ozone molecules formed by α radiation being equal to one-half of the total number of ions formed.

The fact established by Middleton (10), that removal of the polonium some little distance from the barley seedlings abolishes the effect on the respiration rate, is of great interest. If the air current has to travel some little distance from the source of ionization before reaching the biological material the ions have time for recombination; the chemical products

(ozone, &c.) would, however, remain unaltered. Thus there is a clear indication that it is the ions themselves and not the chemical products of ionization which are responsible for the biological effect.

As stated in the introduction, Henrici (7) has suggested that some results on assimilation rates which she obtained are to be explained by changes in the degree of ionization of the air. Her work is confined to a study of *apparent* assimilation; respiration is totally neglected, although, as the results put forward in this paper show, ionization of the air may have a very marked effect on the rate of this process. No support has been found for her claim that assimilation rates may increase four or five times as a result of ionization. It seems unlikely that increases of this nature in the assimilation rates could be produced by a very low degree of ionization of the air.

V. SUMMARY.

Rates of respiration and assimilation of leaves of *Pelargonium zonale* have been studied by a method in which carbon dioxide production and absorption in a closed system is followed by the use of brom-cresol-purple as an indicator. It has been shown that under these conditions accurate results can be obtained, the rates of respiration and assimilation being very regular.

With air ionized by means of polonium to a degree varying from 7.28×10^2 to 3.64×10^2 times that of normal air (assumed to be 500 positive and 500 negative ions per c.c.), a percentage increase of 85.7 ± 7.1 in the respiration rate at 25°C . was observed. After the removal of the polonium there is a definite 'after-effect', the succeeding two hours showing a percentage increase of 28.0 ± 7.3 over the control period.

No effect on the rate of *apparent* assimilation was demonstrable under the action of ionized air. A small depression (3 per cent.) was observed, but it was far below the significant value. A determination in ionized air of the *real* assimilation, in which the increased rate of respiration is taken into account, showed a slight increase (about 5 per cent.), which was again below the significant level. The possibility that ionization of air affects to a considerable degree both real and apparent assimilation is thus still open.

The effect of ionized air on respiration is almost certainly due to the action of the ions themselves, and not to the chemical products (ozone, &c.) produced by the action of the ionizing agent (polonium).

This work was undertaken at the suggestion of Professor V. H. Blackman, of whose help and criticism I wish to express my appreciation.

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On the Shot-hole Disease caused by *Clasterosporium carpophilum* and on the 'Shot-hole' Effect

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With Plates XVII and XVIII and eighteen Figures in the Text.

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I. THE DISEASE.

THE disease here dealt with is that caused by the fungus *Clasterosporium carpophilum*, (Lév.) Aderh. (syn. *Coryneum Beyerinckii*, Oud.), on the almond, *Prunus Amygdalus*, Stokes. Owing to the general use in some countries of the synonym *Coryneum Beyerinckii*, the question of the nomenclature of this fungus is further discussed below.

Historical.

(a) *The disease.* Aderhold (1) published in 1901 a paper which is still the most complete discussion of shot-hole diseases available. In this paper he reviewed former work on the subject, recorded his own observations on the method of formation of 'shot-holes', and listed and described some thirty species of fungi capable of producing this effect. He amplified his

observations in some directions in a paper (2) published the following year upon *Clasterosporium carpophilum*, the most common cause of shot-hole in Europe. In the latter paper he dealt especially with the synonymy of the fungus, and its relation to gummosis of stone-fruit trees.

MacAlpine (12) regarded *Clasterosporium carpophilum* as the most common cause of shot-hole in Australia, and on account of the frequent subsequent appearance of pycnidia of *Phyllosticta prunicola* and perithecia of *Gnomonia circumscissa* on diseased spots, suggested a possible genetic connexion between these fungi. He considered *Coryneum Beyerinckii* as identical with *Clasterosporium carpophilum*, without discussing the relative merits of the generic names.

Trabut (18) gave an account of the occurrence of the fungus in Algeria, assigning to it the name *Coryneum Beyerinckii*. He considered that there were two forms of the disease, one limited to the blade of the leaf, and the other more generalized and attacking young shoots and branches. (This paper was not available to the writer, and it is not known whether any morphological or cultural details of the two forms of the fungus were included.)

Ralph E. Smith (16) described the occurrence of the fungus in America (under the name *Coryneum Beyerinckii*), paying special attention to control by spraying, but also giving some morphological and cultural details.

Finally, C. S. Parker (13) has published an account of the *Coryneum* blight, as it is frequently named in America, which adds practically nothing to our knowledge of the disease.

(b) *The 'shot-hole' effect.* Duggar (6) first drew attention to the fact that the shot-hole effect on leaves of species of *Prunus* was produced not only by a number of fungi, but also by spraying with injurious solutions such as copper sulphate, formalin, corrosive sublimate, and others. He concluded that 'the shot-hole effect on plums, peaches, cherries, &c., is a peculiar reaction of the plant to injuries . . .'. No anatomical details were given in this paper other than the statement that sections revealed no special cell development on the part of the host connected with the abscission.

Aderhold (1), as noted above, recorded, discussed, and amplified previous observations on this subject. He distinguished three methods of falling out of the infected tissue, viz. (1) a hole appearing in the centre of the dead disc of tissue, and gradually enlarging until the latter had completely disappeared; (2) the dead disc falling away as a whole, but passively, without the occurrence of any special cell development on the part of the host; and (3) abscission of the diseased tissue as a result of active cell division on the part of the host.

B. B. Higgins (9), in an account of the shot-hole disease of cherries due to the fungus *Cylindrosporium padi*, advanced some views as to the formation of the absciss layer, which will be discussed later.

As far as can be ascertained, no other papers dealing with the details of host reaction in the case of the shot-hole diseases have been published. Several papers, however, dealing with abscission and wound-reaction in general will be referred to during the discussion of the shot-hole effect in the second half of this paper.

Nomenclature of the Fungus.

Clasterosporium carpophilum, (Lév.) Aderh., is the name most commonly used in Europe and Australia, while *Coryneum Beyerinckii*, Oud., is in general use in America. The question as to which name is correct is one that depends upon whether the fungus is placed in the Hyphomycetes or in the Melanconiales.

The different usage in different continents seems to depend partly on a different manner of occurrence of the disease. In America it is the twig blight which is the predominant form, whereas in Europe and Australia it is the shot-hole on the leaves which is more important. It is generally admitted that on twigs the fungus tends to fruit in distinct pustules, with more or less of a stroma, whereas on leaves the fruiting pustules are usually very much smaller, without a pronounced stroma, and thus more hyphomycetous in character. In this way has arisen, and been perpetuated, the difference in nomenclature in the two continents.

The general practice of calling the fungus *Coryneum Beyerinckii* in America seems to date from R. E. Smith's bulletin (16) on the California Peach Blight, published in 1907. As neither Smith nor Parker, who recently wrote on this disease (13), make any reference to the exhaustive discussion by Aderhold (2), in 1902, of the nomenclature of the fungus, it would seem not out of place to give a *résumé* of Aderhold's conclusions.

After a discussion of the published records and various exsiccatae available, Aderhold summarizes the changes in nomenclature of the fungus as follows:

- 1843. *Helminthosporium carpophilum*, Lév. (on peach fruit).
- 1864. *Macrosporium rhabdiferum*, Berk. (on peach fruit).
- 1865. *Helminthosporium rhabdiferum*, Berk. et Br. (change of genus).
- 1876. *Sporidesmium amygdalearum*, Pass. (on stone-fruit leaves).
- 1882. *Clasterosporium amygdalearum*, (Pass.) Sacc. (change of genus).
- 1883. *Coryneum Beyerinckii*, Oud. (in gummosis wounds).
- 1884. *Septosporium Cerasorum*, Thüm. (on cherry fruit).
- 1886. *Helminthosporium Cerasorum*, (Thüm.) Berl. et Vogl. (change of genus).

He then says, 'Finally, I myself, having regard to priority, have already named the fungus *Clasterosporium carpophilum*, (Lév.) Aderh. It

is certainly a debatable point whether the genus *Clasterosporium* or *Coryneum* is more correct. On the twigs, and usually also on the fruit, the fungus customarily appears as a Melanconiaceous one, but on the leaves as a Hyphomycete. Since the whole system of these Fungi Imperfecti is an artificial one, and further, since analogous cases can be numbered by hundreds, it is of no value to contest such questions.'

Aderhold then devotes twelve pages to proofs of the synonymy, (1) by a morphological comparison of all exsiccatæ available to him and his own collections on various hosts and host parts, and (2) by cross-inoculation experiments with *Clasterosporium* cultures from various sources. The latter included material of the original *Coryneum Beyerinckii*, forwarded to him by Oudemans.

This detailed work seems to have escaped the notice of a number of later workers, for besides making no reference to it when naming the fungus *Coryneum Beyerinckii*, they refer to probable identity with *Helminthosporium carpophilum*, Lév., and other synonyms as though such had not been established.

Specimens of the American fungus have been kindly forwarded to the author by Dr. J. R. Weir, in charge of Pathological Collections in the Bureau of Plant Industry of the United States Department of Agriculture. A morphological comparison of these with the Australian fungus has been made, and would indicate that the two are probably identical; a cultural comparison will also be necessary, however, to establish completely this identity.

As additional evidence bearing upon the systematic position of the fungus, the following observations have been made. In the first place all published figures of the fungus which the writer has seen give a very imperfect idea of the manner of spore formation. The spores are usually shown as produced singly at the ends of short branches of the mycelium. Under favourable conditions, however, many spores are abstricted successively from the same conidiophore, which thus acquires a geniculate shape (Text-figs. 1-4, 12). This is immediately reminiscent of spore formation in the genus *Helminthosporium* and other Hyphomycetes, though of course the spore-bearing hyphae in *Clasterosporium* are not specially thickened or in any other way differentiated into conidiophores distinct from the mycelium. Indeed, a hyphal branch, after successively abstricting a number of spores in the manner described, may temporarily resume growth as a hypha and then abstrict more spores as a conidiophore.

This morphological feature, together with the cultural peculiarities described below, viz. (1) great preponderance of single conidiophores instead of the aggregations of conidiophores usually characteristic of Melanconiaceous fungi, and (2) formation of sectorial mutants in single-spore cultures in a manner very comparable to that described for certain species

of *Helminthosporium* (5, 17), indicates strong affinities with the Hyphomycetes.

Further, a careful examination of the stromatic fruiting pustules which the fungus forms on its host also points, in the opinion of the writer, to a greater Hyphomycetous affinity than a Melanconiaceous one. In the characteristic acervulus of the Melanconiaceae spores are usually formed in considerable numbers before the epidermis is ruptured, whereas in the fungus under consideration it is seldom until after the epidermis is ruptured that spores are formed from the surface of the stroma. Small stromatic aggregations of hyphae giving rise to conidiophores, at least of the size of those of the shot-hole fungus on leaves, are also well known among the Hyphomycetes, and in the genus *Fusarium*, for example, these fruiting tufts may attain a size considerably greater than is ever reached by the pustules of *Clasterosporium carpophilum* on twigs.

Even were it to be conceded, however, that the fruiting pustules of this shot-hole fungus had a distinct Melanconiaceous character, it could not be denied by any one who had worked with the fungus in culture that it has very many Hyphomycetous affinities, and is at least on the border-line between the two groups. This was expressly pointed out by Aderhold, who has done more work on the fungus than any other investigator, and it would seem presumptuous to disregard his naming without adducing any additional evidence why a Melanconiaceous position should be adopted. On the contrary, it is believed that the morphological and cultural points enumerated above strengthen Aderhold's contention, and no alteration in his nomenclature would appear justified unless supported by a detailed comparison of a considerable number of species of the genera *Coryneum* and *Clasterosporium*. Finally, it is admittedly desirable to have one name for one organism, and it is believed that the above considerations undoubtedly indicate *Clasterosporium carpophilum* to be the more fitting name for this fungus.

It should perhaps be noted that in 1917 Elliot (7), discussing the taxonomic characters of the genera *Alternaria* and *Macrosporium*, found that of the four species of *Macrosporium*, from which the genus was created, two belonged to *Alternaria*, and the remaining two were later placed in the genus *Clasterosporium*. He therefore claims that the name *Clasterosporium* must give way to *Macrosporium*. Sarciniform, non-beaked spores usually placed in *Macrosporium*, he says, really belong to *Stemphylium*. The genera *Macrosporium* and *Clasterosporium* have now been established so long, however, and have so many well-defined species, that it is doubtful if this change will ever be accepted.

The Fungus in Culture.

Aderhold (2) has already described the growth of the fungus in considerable detail on a number of substrata, including sterilized leaves and fruits, fruit-juice and other gelatines, cherry and peach gum, &c. Parker (13) noted that spore formation was much more abundant on prune agar than on 3 per cent. dextrose agar. In the present work corn-meal agar and Quaker oats agar were found to be the best media for studying growth and spore-formation, the latter occurring much more sparingly on potato agar, Dox's agar, and other agars.

Spore-formation usually occurs from single conidiophores or fertile hyphal branches, which abstrict successively a considerable number of spores in the manner described above. Examining a vigorous culture from the edge towards the centre, one finds first, at a short distance from the growing margin, young spores being formed singly at the ends of hyphal branches. Farther in, small heaps of spores are found over the surface of the culture; but these are not formed from tufts of conidiophores, as in many Melanconiaceae fungi, but are the spores which have been successively abstricted from one conidiophore. Still farther in, more conidiophores may have arisen, and spore abstriction have proceeded so far, that the whole surface of the culture is covered by a continuous layer of abstricted spores. In vigorous cultures on corn-meal or Quaker oats agar, after a few weeks scattered, much denser heaps of spores may be seen towards the centre, visible to the naked eye as raised, black lumps somewhat smaller than a pin's head. These heaps are formed from dense aggregations of conidiophores, the early stages of which are figured by Aderhold. They approach in structure the fruiting pustules which occur on twigs, and are the only fruit form which may possibly be regarded as an acervulus.

Another feature is the fairly frequent occurrence in single-spore cultures of sectorial 'mutants' or 'saltants', distinguished mainly by a more sparing or more abundant fruiting of the single conidiophore type, which is the predominant form on all artificial media (Pl. XVII, Fig. 13). Subcultures from such mutant sectors and from the parent colony retain the habit of sparing or abundant fruiting which characterized them in the original culture, though further 'mutation' may occur. This peculiarity of growth has lately been described as occurring frequently in some species of *Helminthosporium*, notably *Helminthosporium sativum* (5, 17). In this connexion the observation of Aderhold, which has frequently been confirmed in the present investigation, that of a number of single-spore colonies of *Clasterosporium carpophilum* a few often remain sterile or relatively so, is of some interest.

Germination of the Spores and influencing Factors.

Viability of spores. The viability of the spores was tested by keeping infected leaves on which the fungus was fructifying in a dry condition in a cardboard box in the laboratory, and testing the germination at intervals of one month. For the first fifteen months over 90 per cent. of the spores germinated in distilled water, mostly within two hours. After that, however, the percentage germination rapidly fell off, and at the end of eighteen months, when the experiment had to be discontinued, less than 20 per cent. of the spores germinated. Parker (13) conducted such an experiment, but abandoned it at the end of six months, when the spores were still germinating vigorously.

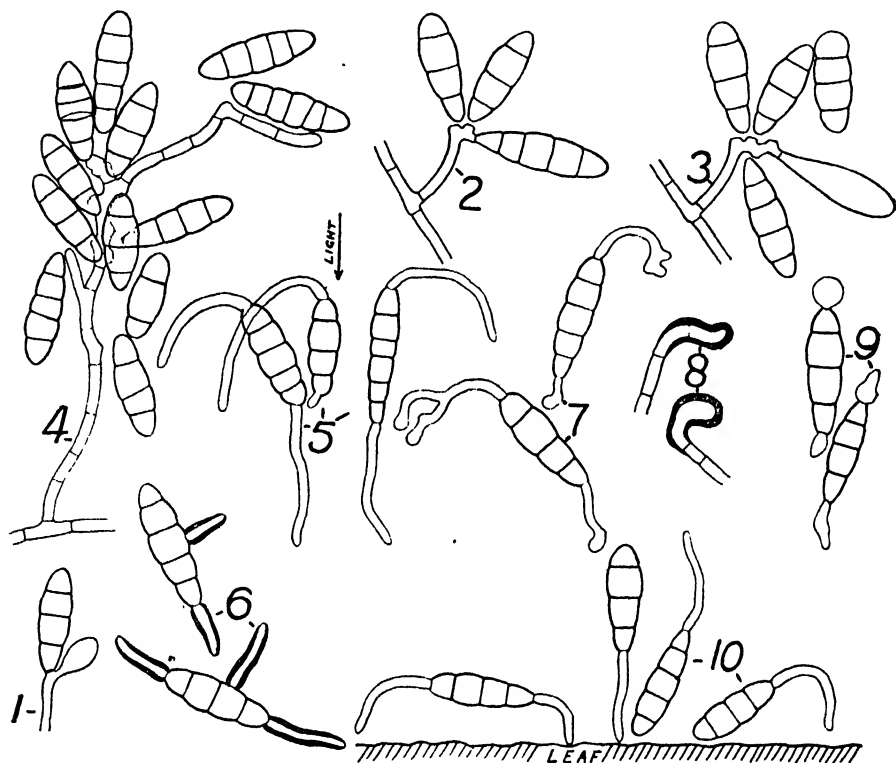
Germination of the spores. Germination of the spores will occur almost immediately after formation, when removed from the vicinity of the parent mycelium and placed in distilled water or nutrient media. Germination is very rapid, the beginnings of the germ-tubes being easily visible after one hour, even in the case of spores kept dry for many weeks. As many as ten germ-tubes may be formed from one spore in nutrient media, but in water from one to three are usually formed.

Mucilaginous sheath round germ-tubes. The germ-tubes are surrounded from the commencement by a mucilaginous sheath (Text-fig. 6), which fixes them to the substratum upon which they are germinating. This may be demonstrated in the usual manner by staining with dilute gentian violet, or by covering with dilute Indian ink. The most suggestive experiment in this connexion is to subject the germinating spores to a fairly strong current of water, when it is found almost impossible to wash them from the substratum on which they have germinated.

The thickness of the mucilaginous sheath which surrounds the germ-tubes varies considerably in different lots of material, and is sometimes so thin that it is not demonstrable by staining. This seems to be correlated in some way with the history of the development of the spore, it being often, but not always, found that spores formed on the host plant produce a thicker mucilaginous sheath round their germ-tubes than those formed in the moist atmosphere of a culture-dish.

Aderhold has already noted the fact that the germ-tubes may sometimes swell up into appressoria-like organs at the ends, and remarked that there appeared to be no constancy in their formation, even when contact with the substratum was demonstrable. In the present experiments it has been found that these swellings appear more frequently when the germinating spores are exposed to bright light (Text-fig. 7); even then, however, many spores show no signs of forming them. When these appressoria-like swellings are formed, they are always surrounded by more mucilage than the rest of the germ-tube (Text-fig. 8).

Negative heliotropism. When exposed to unilateral illumination the germ-tubes of germinating spores grow away from the light; those which on account of their point of origin are at first forced to grow out towards the light soon turn, often through 180° , to grow directly away from it (Text-fig. 5). In the case of germ-tubes produced by spores germinating



TEXT-FIGS. 1-10. 1. Abstriction of first spore from a conidiophore in hanging-drop culture. 2, 3. Successive stages in spore abstriction from another conidiophore. 4. Illustrating resumption of hyphal growth by a conidiophore. 5. Spores germinating under the influence of unilateral illumination. 6. Mucilaginous sheath round germ-tubes. 7. Appressoria-like swellings on the germ-tubes of spores germinating under the influence of unilateral illumination. 8. More abundant mucilage round appressoria-like swellings. 9. Abnormal germination in chemotropic experiments with pieces of ivy leaf. 10. Chemotropic response on the part of some germ-tubes in the vicinity of a fragment of almond leaf. $\times 375$ (Fig. 8 slightly larger).

in distilled water, this property is retained almost as long as the germ-tubes grow. In the case of spores germinating in a nutrient solution, however, although the germ-tubes on their first appearance are negatively heliotropic, branching soon sets in, and the hyphae grow in all directions, apparently uninfluenced by the direction of the light.

Chemotropism. Experiments were tried to see if the germ-tubes gave any chemotropic response, but they were inconclusive. In many of them no trace of such response could be detected, but in others there was a marked

turning of some of the germ-tubes towards the piece of host leaf. Such a turning as is illustrated in Text-fig. 10, for example, never occurs when spores are germinated in water alone; it seems beyond doubt that such germ-tubes have exhibited a definite chemotropic response. It only occurred, moreover, in the case of spores in close proximity to the section of host tissue. In the experiments in which it did occur, however, there were always some spores equally close to the host leaf which exhibited no trace of such response. This behaviour is evidently parallel to that of many other fungi for which a chemotropic reaction has been claimed. It has frequently been recorded for chemotropic experiments (e.g. 8), that some hyphae showed a distinct turning, whereas others equally favourably situated showed none whatever. These experiments only emphasize the very indefinite state of our present knowledge of chemotropism of fungi.

Infection of the Host.

Aderhold has already stated that infection may occur by direct penetration of the epidermis, and this was easily proved by sowing spores on the uninjured upper epidermis of almond leaves, which is devoid of stomates; abundant infection always occurs. Parker (13), however, found no infection through the upper surface of peach and apricot leaves, but the methods used were too crude for much reliance to be placed on the results. He described and figured the entry of germ-tubes through the stomates, but if the fungus is able to penetrate the uninjured cuticle as demonstrated in the present paper, it is not likely that stomatal infection frequently occurs.

Methods. To follow the early stages of penetration, spores were sown fairly thickly on the upper epidermis of freshly picked almond leaves kept moist in Petri dishes in the light; part of this material was fixed in half-strength Flemming's solution, and in Carnoy's solution after the lapse of 4, 8, 12, 24, and 48 hours respectively. Difficulty was experienced in sectioning all the material embedded by the xylol method owing to the cuticle becoming very brittle, and also crystals of calcium oxalate frequently being carried through and breaking it. The cedar-oil method gave more satisfactory results, and sections were cut 5-8 μ thick, and stained with haematoxylin and erythrosin or orange G, and with Flemming's triple stain.

Penetration of the cuticle. It was found that penetration of the cuticle occurred by means of a narrow infecting hypha springing from the germ-tube, appressed and held to the outer surface by its mucilaginous sheath. The germ-tube may or may not have dilated on the outside to form a small appressorial pad. It is probable that penetration occurs as a result of pressure alone, as has been proved for several other fungi. Once penetra-

tion of the cuticle has occurred the narrow infecting hypha dilates again on the inside into a hypha of normal or frequently of increased diameter. At the same time the subcuticular layers of the epidermal cell-walls begin to swell up, probably under the action of enzymes excreted by the parasite (Pl. XVII, Figs. 1, 2; Pl. XVIII, Figs. 5, 6). This swelling often shows up a pronounced stratification in what originally appeared a homogeneous cellulose wall. (It should be noted here that these stages of penetration were obtained on detached leaves in Petri dishes; under natural conditions it is unlikely that moisture relations would permit of such a large swelling of cellulose walls as is shown in some of the figures.) The cuticle also frequently becomes loosened a little from the cellulose wall beneath, round the point of infection. It may be that this loosening gives the infecting hypha a little play, so that the occasional bulging of the latter just below the cuticle, noted above, may be due to its swelling out in this small space before it is able to push its way into the softening cellulose wall beneath. The loosening of the cuticle becomes very marked when a large number of spores are sown on the surface of leaves kept moist in culture dishes; after three or four days the cuticle can be easily lifted off without resistance for just as great a distance as the fungus hyphae have grown beneath it. Beyond the limit of the hyphae it sticks tightly and breaks immediately on any further pulling.

Growth within the host. Once inside the host the fungus hyphae branch, and grow in different directions. Branching, however, is sparing, and growth is very slow compared with that on nutrient media. Some of the hyphae grow in the substance of the cellulose walls for comparatively long distances, but others grow straight through the centres of cells, and penetrate the opposite walls apparently quite easily. The cells of the host die some little distance in advance of the fungus hyphae. At first practically no change can be detected in the appearance of affected cells, unless it be that the chloroplasts become very slightly paler green, but the lethal effect can be demonstrated by plasmolysis with strong salt solution. The fact that some alteration has occurred, moreover, is frequently evident in stained slides by the different staining reaction of cells apparently still normal, but situated near the infection point.

Beginning from the infection point as a centre, the cells soon commence to show pathological changes, involving a browning and a shrinking until all detail is obliterated. The fungus mycelium remains small in amount, and in sections of infected tissue only occasional small pieces of hyphae crossing between the dead cells are visible. It is not until spore-formation commences that any amount of mycelium is easily seen, and then it is only in comparatively small sub-epidermal aggregations at the points where groups of conidiophores are to arise. Conidia are not formed until the conidiophores have ruptured the epidermis (Text-fig. 12).

The size which the circle of tissue killed by the fungus may attain varies from less than the size of a small pin's head to half a centimetre or more. This variation in size is due to the fact that the speed at which the host cells react, and also the closeness of this reaction to the injured tissue, are both variable quantities. The details of reaction are discussed in the following sections, but the result is always the same, viz. that a limit is set to further growth of the fungus, the injury often being abscised in the familiar shot-hole manner.

Host Reaction.

Infection may occur on leaves, fruit, and on one-year-old twigs. In all cases there is a definite reaction on the part of the host, which leads to the segregation of the fungus in a small circular area of tissue, which in the majority of cases sooner or later falls away, leaving the host-plant free from infection. In the case of leaves this falling away of the infected discs has been described as occurring in three different ways, namely, active abscission, passive falling out as a result of drying, aided perhaps by subsequent growth of the leaf surface, and progressive powdering away from the centre. No detailed anatomical description of these processes has been found in the literature, and the significance of the differences which exist between them has therefore not been appreciated. The following section aims at giving an account of the anatomical changes involved in host reaction, and some physiological aspects are discussed later in the section on the 'shot-hole' effect.

Reaction of leaves, fruit, and twigs. Previous to the work of Aderhold, three methods of falling out of the infected discs of leaf tissue had been described. Firstly, in the case of spots caused by *Septoria erythrostoma*, Frank had noted that vigorous callus-formation commenced in the healthy tissue round the affected spot, the latter soon being actively thrust out, or abscised. Secondly, in the case of spots caused by *Clasterosporium carpophilum*, Frank observed the dead tissue to dry and crumble away from the centre, where a small hole first appeared, and enlarged until all the dead tissue had fallen away. Thirdly, Duggar described a passive falling out of the dead discs as a whole, without the development of any special separation tissue, and presumably because of contraction through drying. Aderhold (1) confirmed all these observations, and considered that the different methods of abscission did not depend upon the parasite, but upon external conditions of which he supposed the stage of development of the leaf to be the most important, with conditions of nutrition and the atmospheric humidity also playing a part.

Of the three methods of falling away discussed above, the progressive crumbling away of the dead tissue from the centre was not seen in the

present investigation. The two methods whose development has been followed, however, probably represent the remaining two described by Aderhold, the third (namely, crumbling from the centre) probably being a special case of one of the others.

Macroscopic changes. In very young leaves the infected tissue is invariably abscised. The host reaction sets in very rapidly, and very close to the point of infection, so that the abscised tissue may be less than the size of a small pin's head. Subsequent growth of the leaf surface usually leads to a considerable enlargement of the holes thus formed, so that in this case the final size of the hole is no criterion of the size of the infected spot.

In older leaves the course of events is less rapid, and thus more easily followed. On the whole, direct abscission is the more common method during spring and early summer when the leaves, though fully grown, are still relatively young. The cases in which infected tissue is not actively abscised, but either remains *in situ*, or falls away as a result of shrinking, are more common in mid and late summer, when the leaves are more mature.

The macroscopic changes occurring in the case of direct abscission may be described as follows: Round the spot of tissue killed by the fungus, and usually at a distance of from 1 to 2 mm., a thin, definite line becomes visible in the mesophyll, which from the under-side of the leaf appears a darker green, as though the tissue there had become waterlogged, but which appears as a narrow translucent line when the leaf is held up to the light. The ring of apparently healthy leaf surface which is thus cut off on the inside of this line, together with the infected tissue, soon becomes a slightly paler green than the normal leaf surface, but is quite turgid, and unaffected by the fungus. Often within a day or two of the first appearance of the line, though sometimes a week or more is required, if the leaf be examined with a hand-lens it is seen that the cuticle of both upper and under surfaces is ruptured in the exact track of the line. This break in the cuticle follows the line so exactly that it appears almost as if the cuticle had been split round with a very delicate knife. The cells beneath soon begin to bulge out in this break as though cell division and growth were proceeding within, and very shortly it is seen that the cells are completely separating along the track of the line, the central disc being definitely abscised. The disc, when it finally falls away, is found to be too large to fit back into the hole from which it fell. This is because of the continued growth of the leaf cells at the margin of the hole; in a very moist atmosphere these may swell up to a considerable size, giving the margin that glistening mealy-white appearance peculiar to other absciss layers. The abscised disc consists of a central brown portion killed by the fungus, surrounded by a border of pale green, still living tissue. If the disc be kept

moist, the fungus continues its growth, killing the whole tissue as far as the margin.

The time required for these developments is very variable. The variety of host plant, the age of the leaf, and the weather conditions are all interacting factors.

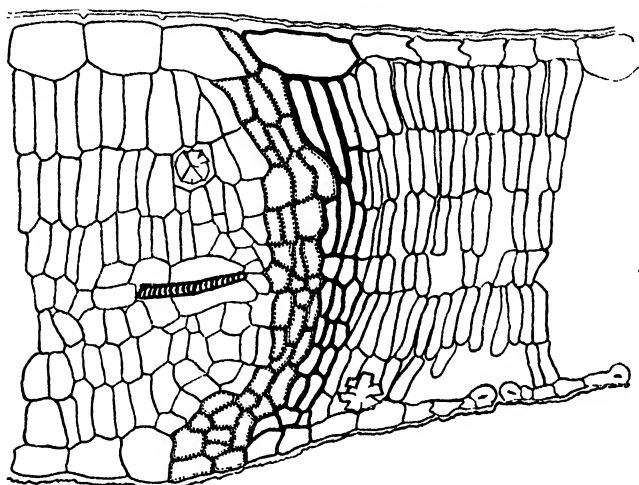
In those cases in which abscission does not occur the spots do not extend indefinitely. Extension appears to be blocked by some internal barrier erected by the host, for at the margin of the spots, when examined with a lens, a yellowish-green line can often be seen surrounding them. This reminds one somewhat of the absciss line described above, though much less distinct, and in close contact with the dead tissue. Later the dead discs often tear away at this junction through drying and shrinking, aided perhaps by increase in the leaf surface, but they may remain in the leaf indefinitely.

Microscopic changes. Case I. Abscission. The first noticeable anatomical change is a swelling up of the cells in a narrow zone at some distance from the infected spot. Both palisade and spongy parenchyma cells are involved, and complete occlusion of intercellular spaces results. This is undoubtedly the explanation of the naked-eye appearance of the future absciss line as a rather dark, apparently waterlogged line when viewed from the under-surface of the leaf, and as a pale green, semi-transparent line when the leaf is held up to the light. The distance from the injured tissue at which this absciss line arises is variable. Sometimes the line arises only three or four cells distant from the injured tissue, while at other times from ten to twenty or more healthy cells intervene. These cells, which, though apparently a part of the normal healthy leaf surface, are yet destined to be abscissed along with the injured part, show slight changes. They may still be plasmolysed by strong salt solutions, and so are presumably not dead, but the chloroplasts especially seem rather paler green, and both chloroplasts and protoplasm in them gradually diminish in bulk.

Simultaneously with the swelling of the cells along the future absciss line at least three physiological changes are proceeding. In the first place starch begins to accumulate in this region, sometimes more abundantly than at others. Secondly, some lignification occurs of the walls of those cells of the occluded region which are nearest to the inside, i.e. to the infected tissue. Thirdly, there is a progressive disappearance of the chloroplasts and an increase in the protoplasm of the cells of the occluded zone just without the lignified circle; the vacuoles disappear, the nuclei enlarge, and these cells take on all the characters of meristematic cells (Pl. XVII, Fig. 3). The epidermal cells, as well as those of the palisade and spongy parenchyma, are frequently involved in this process of rejuvenation. Cell divisions begin to occur, often obliquely in the epidermis, but in a plane tangential to the diameter of the infected spot in the palisade and spongy parenchyma.

Soon after this the cuticle breaks, probably as a result of tension, as is discussed later. The cells within begin to bulge out, and separate along their middle lamellae beneath the break in the cuticle (Pl. XVII, Fig. 4). This progresses until the central disc is forced out and drops away.

Owing to the fact that separation occurs along the middle lamellae the cells left at the margin of the hole are bulged out and spherical, and rarely have torn cell-walls. In a very moist atmosphere the swelling of these cells



TEXT-FIG. 11. Section of almond leaf illustrating the layer of wound-cork isolating the infected tissue in a case where abscission did not occur. Suberized cells dotted round the margins; lignified cells heavily outlined. $\times 250$.

may go on for some time, until they are ten or twenty times the volume of the mesophyll cells. Soon after the cuticle breaks suberization of the walls at this abscission line commences, and subsequent divisions of the meristem layer lead to the formation of a few layers of brick-shaped cells, which become suberized and slightly lignified, and protect the exposed surface in the manner of wound-cork.

(The above changes occur in an almost parallel manner in the leaf of the cherry-laurel, and are figured for this in the second half of the paper.)

Case 2. No abscission. It was remarked above that during spring and early summer infections are practically always abscised, but that as the leaves become older, and the summer hotter and drier, abscission sometimes fails to occur. In these cases the circle of infected tissue either remains permanently in position, or tears away at the margins, usually owing to shrinking, and may finally drop out.

When these cases were examined microscopically it was seen that the sequence of changes in the early stages was almost parallel to that occurring when abscission is the result. There is a blocking of the intercellular spaces of the mesophyll, but it is much less extensive and often not obvious

to the naked eye, and it usually arises closer to the injured tissue than is the case where abscission is to occur; there is lignification of cells on the inner side of this occluded zone, and there is the assumption of meristematic activity by the cells immediately without the lignified layer. These changes, however, proceed without the turgidity and obvious activity characteristic of those preceeding abscission. At this point, instead of the epidermis rupturing and the cells separating along their middle lamellae, suberization of the meristem-formed cells takes place. The central tissue is thus cut off from its water-supply, and the cells soon die right up to the suberin-lignin block (Text-fig. 11). Whether the dead disc of tissue falls out, leaving a 'shot-hole', depends upon whether it shrinks and thus 'tears itself out', or whether slight subsequent growth in the leaf surface occurs leading to the same result; in either case it is purely a physical process.

In view of the statements by other investigators, as to 'passive falling out' of infected discs, it seems opportune to summarize some of these statements here. Aderhold (1) quotes Duggar (6), who said: 'I anticipated finding some unusual cellular development along the line of abscission [speaking of shot-holes in general], but free-hand sections of various stages indicate that advantage is taken of the normal leaf development. The cells which make up the minutest ramifications of the veinlets seem to afford a place through which a break most readily occurs with least injury to delicate parts adjacent.' Aderhold continues: 'This explanation of the occurrence, which I have consequently set down word for word, in its full extent, is difficult to understand. One may assume from it, however, that Duggar regarded the falling out not as the consequence of the formation of a separation-tissue, the process being an active abscission, but as a passive breaking away of the dead portions.' Later, in describing the three methods of falling out of infected discs of tissue, he says under No. 2: 'The dead disc falls out as a whole, but passively, without the formation of any abnormal tissue development. This case, which Duggar evidently had before him, occurs in only slowly growing or fully grown leaves, in normal, neither too dry nor too moist atmosphere. The piece of leaf falls out, according to my own conception, because tissue tensions set in as a result of its drying, which finally lead to a tear at the border between the pliant, living leaf surface, and the hard, brittle dead tissue. . . .'

If such passive dropping out does occur, it must do so fairly rapidly, as otherwise there would be nothing to stop the continued extension of the fungus into the leaf. Since infected spots often remain in position for a considerable time, however, or even indefinitely, it is obvious that there must frequently be something which sets a limit to further growth of the fungus. In all cases examined in connexion with the present work, this limiting factor, when direct abscission did not occur, was found to be the development of cork described above. It is therefore suggested that in at

least many of the cases which have previously been described as a passive falling out, the host had already formed a few layers of cork round the infected spot.

Case 3. Crumbling away from the centre. This case, perhaps better described as a progressive disappearance of the infected disc from the centre outwards, producing a continually enlarging hole, was described by Frank and Aderhold, but has not been seen in South Australia. It seems most probable, however, that the anatomical changes are the same as those described for Case 2, whereby further growth of the fungus is restricted by the formation of a suberized and lignified barrier round the spot. If, then, weather conditions were such that the dead disc did not shrink and tear out, and there was sufficient moisture for continued activity of the fungus, it is possible that the central tissue might slowly be dissolved by the action of the fungus enzymes, leading to the formation of a gradually enlarging hole.

Aderhold, in endeavouring to obtain a higher fruiting form of the fungus, made an experiment in which he collected some abscised discs and exposed them in a cotton bag to the action of the weather during the winter; after some months he found only little circles of tissue remaining, and suggested also that the continued action of the fungus led to progressive dissolution of the tissue from the centre.

According to this view, it is not surprising that this type of disappearance of the infected tissue has not been observed in South Australia. The very dry summer climate usually leads to contraction and tearing out when abscission does not occur, and in those cases where the discs do remain in place there is usually not sufficient moisture present for continued activity of the fungus. A tendency for a hole to appear in the centre of infected discs has been noticed in specimens sent from Victoria, which has a cooler and moister climate.

Reaction of fruit and twigs. These cases may be mentioned, though no special study was made on them.

Infection of the fruit of the almond by *Clasterosporium carpophilum* frequently occurs while the pericarp is still green; the reaction, however, involves gummosis, bringing in factors beyond the scope of the present paper. The reaction of apricots to the same fungus is not complicated in this way, however, and may be briefly described.

As in the case of leaves, reaction to infection is much more immediate, and close to the seat of injury, in young fruit than it is in fully grown, or nearly fully grown, fruit (Text-figs. 13, 14). In the latter case there is time for the fungus to kill more tissue before reaction sets in, so that the scabs are usually somewhat larger. Meristem formation commences in the tissue round the infected spot, and a cork cambium is formed which may cut off very numerous rows of cells. These early become suberized, thus cutting off the water-supply to the infected tissue and leading to the

formation of a dry scab. The contraction of the drying tissue usually leads to its tearing away at the edges, and this frequently proceeds so far that the whole scab drops off, leaving a clean surface completely protected by wound-cork. Continued growth of the fruit probably assists materially this complete dropping away, which does not occur so frequently in later formed scabs.

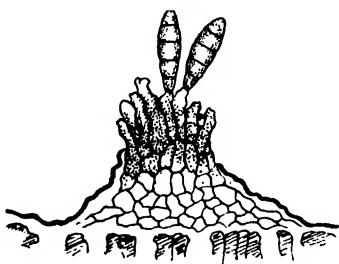


FIG. 12.

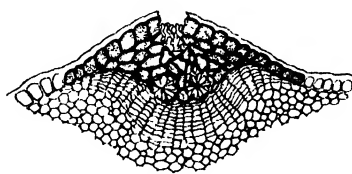


FIG. 13.

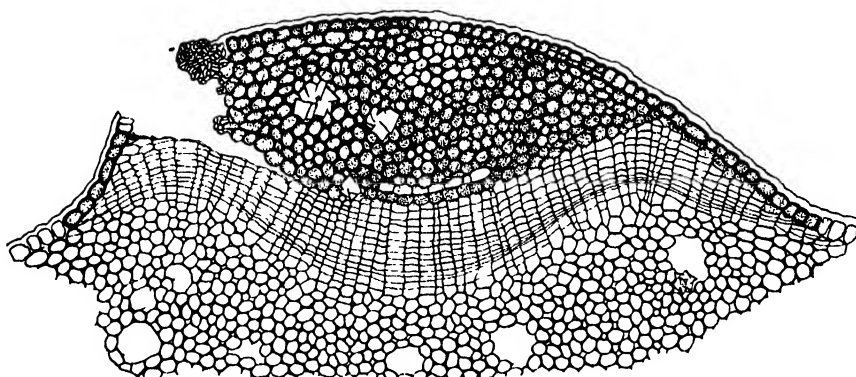


FIG. 14.

TEXT-FIGS. 12-14. 12. Fruiting pustule of *Clasterosporium carpophilum* on leaf kept for three days in moist dish; illustrating geniculate shape of conidiophores due to abstriction of successive spores, and small stroma. $\times 281$. 13, 14. Reaction of almond twigs to infection by *Clasterosporium carpophilum*, showing small and large scabs, and commencement of tearing away in larger scab. $\times 87$.

A similar cork cambium is formed round twig infections, and here also the scabs may completely drop away, though this is not so frequent as on fruits. Twig infections frequently remain in place for a year or more, the mycelium of the fungus remaining viable, and producing a crop of conidia in spring which probably serve to initiate the shot-hole infections of the new season.

II. THE 'SHOT-HOLE' EFFECT.

The above investigation was undertaken because no detailed description of host reaction in the case of shot-hole diseases was to be found. It became evident as a result of the work that the changes involved were closely

parallel to those described by Blackman and Matthaei (4) for the reaction of leaves of cherry-laurel (*Prunus Laurocerasus*) to traumatic stimulation. It is most probable that they constitute a type of reaction characteristic of *Prunus* species in general, for the anatomical changes for shot-holes in peach and apricot leaves were found to agree in all essentials with those on almond leaves, when examined in connexion with the present work. On account of the close parallelism of the reaction in the two cases, some further work was done with cherry-laurel leaves, because of their larger size and more resistant qualities, in an endeavour to determine more exactly the influence of different factors upon this type of leaf reaction. Since during the course of this work one or two points not noted by Blackman and Matthaei were observed, a brief account of the anatomical changes as they occur in cherry-laurel leaves is here given.

Anatomical Changes.

Blackman and Matthaei describe the swelling up of the mesophyll cells to form an occluded zone, but do not note the fact that the walls of several rows of cells on the inside of this zone (i.e. nearest the injured tissue) become lignified (Text-fig. 15). This lignification presents a sharp face to the still healthy leaf-tissue, ending so abruptly that in a median section one palisade cell may be seen to have strongly lignified walls, while its neighbour is entirely free from such. This lignification usually involves a zone of from two to eight cells, those towards the inside being progressively less changed; epidermal cells as well as those of spongy and palisade parenchyma are changed. At this stage, then, the injured tissue is surrounded by a zone of lignified cells (formed at some distance out in the healthy leaf-surface), presenting on the outside an abrupt face against which the leaf-cells are losing their assimilating nature and acquiring meristematic characters.

It would seem quite likely that such a lignified zone would play an important part in the process of abscission. The actual absciss line arises immediately outside this zone. Blackman and Matthaei say that a row of cells divides into two, and that the cells so formed separate along their middle lamellae. In the present investigation no evidence was obtained of such a regular preliminary division right across the leaf thickness. It appeared rather that a number of divisions might have taken place in one part and none in another. When separation along the middle lamellae takes place, however, it occurs between the two layers of cells immediately outside the lignified zone (Text-fig. 16).

It is in the breaking of the cuticle, however, that this zone may be of most importance. No indication of any softening of the cuticle, or of any dissolution of it producing a thinner place, has ever been observed, even in sections of parts where it is just about to burst. In this connexion it

should be remarked that on examining with a strong hand-lens a leaf where the cuticle above the absciss line is on the point of breaking, it will be seen that the line along which the break will develop is in a very slight depres-

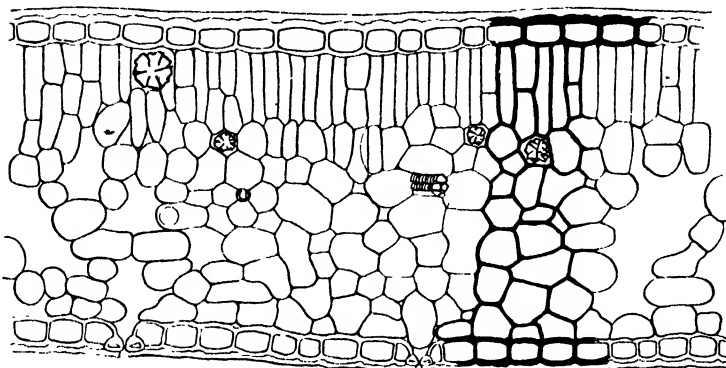


FIG. 15.

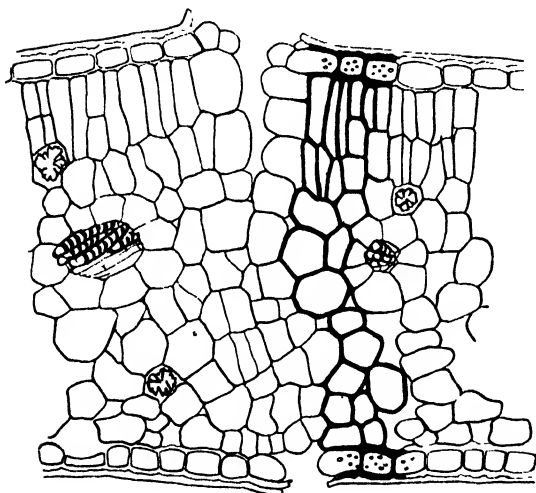


FIG. 16.

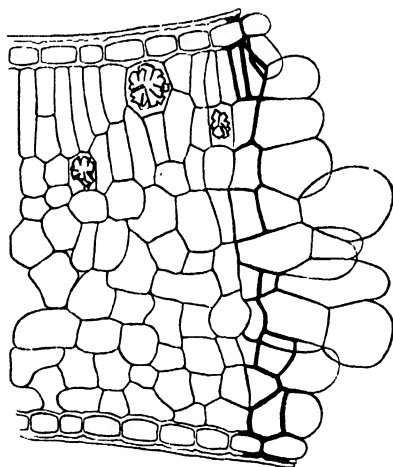


FIG. 17.

TEXT-FIGS. 15-17. Three stages in the process of abscission in cherry-laurel leaves. 15. The occluded zone, with lignified zone towards the injured tissue. 16. The epidermis ruptured and separation occurring between the middle lamellae of the two layers of cells immediately without the lignified zone. Suberization is just commencing in the cells on the healthy side at this stage. The lignified zone drops away with the injured tissue when abscission is complete. 17. The margin of the healthy leaf after abscission is complete. Suberization has occurred in two or three cell-layers at this stage, and some lignification also. Suberization not shown; lignification illustrated by heavy outlines. The injured tissue is towards the right in all cases. $\times 137$.

sion, not a bulge. (There is usually a rather extended bulge covering the width of the occluded zone.) On cutting sections this depression cannot be distinguished, and it would thus seem that it may be an actual thin place due to tension. In the development of such a tension the lignified zone described above might be an important factor. It would provide a rela-

tively rigid wall against which the pressure of the developmental changes proceeding without could exert a force which would ultimately snap the cuticle.

Few other points in the process call for remark. Suberization usually begins very soon after the cuticle has broken. The necessity of oxygen for this process is now generally recognized. Some lignification of the cells at the scar takes place as well as suberization. The swelling up of the cells at the absciss line may proceed in a very moist atmosphere until they are many times the size of mesophyll cells. This swelling up is accompanied by a diminution in the quantity of starch that was accumulated in the neighbourhood of the absciss line, and is thus very probably brought about in part by the increase in pressure due to its hydrolysis. A preliminary accumulation of starch was not noted by Blackman and Matthaei; this is a characteristic of other well-known cases of abscission (e.g. Lloyd (11)). There is also a marked parallel between this abscission in cherry-laurel leaves and some of the types of leaf-fall described by Lee (10).

(In the microchemical tests suberin was demonstrated by treating sections with strong sulphuric acid, when everything dissolves except the two cuticles and the suberin protective layer uniting them; also by treatment with Sudan III, when the cells of the protective layer take the red stain in a slightly different way from the two cuticles. Further distinguishing of suberin from cutin was not attempted. Lignin was demonstrated by treating sections, after clearing, with phloroglucin in hydrochloric acid, and with aniline sulphate.)

Factors influencing Abscission.

Blackman and Matthaei state that abscission did not occur when they wounded leaves on the living bush; they figure a cork-cambium which developed round these wounds, shutting off the injured tissue. However, whether abscission occurs or not depends merely upon certain conditions discussed below, and leaf-wounds made on the living bush will frequently be abscised in typical manner. The following experiments support this view:

In February, 1925, a number of leaves on a cherry-laurel bush in the Adelaide Botanic Gardens were wounded by three knife slits on each side. Then on one half of each leaf 2N hydrochloric acid was rubbed. This has the effect of killing from one to two millimetres of mesophyll tissue round the cut, and of making the absciss line arise considerably farther within the leaf-surface. Less than three weeks later the injured tissue on the side treated with hydrochloric acid was completely abscised, whereas the wounds not so treated were closed by a 'cork-cambium'.

However, on two of the leaves which were removed from the bush

after one week and placed in water under a bell-jar in the laboratory, the injured tissue on both sides was abscised. Wounds made on other leaves on the bush by burning them with a hot piece of iron about four millimetres in diameter were also abscised.

In February, 1926, this experiment was repeated, but in this case there was no abscission, even of the tissue killed by hydrochloric acid (except that in one or two places the lower epidermis had been burst along the track of the abscission line, without separation having proceeded any farther). Both injuries were thus closed by a definite 'cork-cambium'. In this case, also, when two leaves were removed to a moist jar in the laboratory after the first week, abscission of the injury occurred on both sides.

This experiment, repeated again later in the year after rain had fallen, gave the same result as that of February, 1925, viz. abscission of the injuries when the meristem was formed some little distance within the leaf-surface, as a result of killing a larger number of cells, but no abscission and a protective cork layer when the meristem was formed very near to the cut.

It would seem justifiable to draw the following conclusions from these experiments:

(1) *That in a moist atmosphere, with a good water-supply to the leaf, all injured tissue will be abscised.* In this connexion the removal of leaves to the laboratory is not free from objection. It would have been preferable to surround leaves still on the bush with some apparatus for keeping the atmosphere thoroughly moist, and see whether abscission of small as well as large injuries would have occurred. The experiments were conducted under some difficulty as it was, however, for the only cherry-laurel bush available was nearly four miles from the laboratory. Experiments on small branches kept in the light in a greenhouse gave similar results to those on single leaves, however, and there seems little doubt that the above conclusion would hold for the living bush also.

(2) *That in a moderately dry atmosphere, with a good water-supply to the leaf, abscission will or will not occur according as the meristem arises some distance within the leaf lamina. or near to the edge of the injury.* It would seem probable that it is primarily a question of water-supply and the development of sufficient pressure at the meristem. When the latter arises close to the injured tissue, which in a dry atmosphere dries out rapidly, it is probably subjected to a continual water loss through evaporation until suberization of meristem cells has occurred, and abscission is then out of the question. On the other hand, when the meristem can be made to arise some distance within the healthy leaf lamina, it is protected for some considerable time from excessive water loss on the injured side, and sufficient water-pressure can be developed to give rise to abscission.

This was the condition of affairs in the experiment of February, 1925. A fall of six inches of rain had occurred a fortnight previous to the experi-

ment, so that a good water-supply was available to the roots though the atmosphere had had time to become fairly dry.

(3) *That in a dry atmosphere, with a poor supply of water to the roots, abscission will not occur at all, the meristem forming cells which become suberized and form a protective cork-cambium alone.* Again it would seem probable that the explanation is inability to develop a sufficient water-pressure for abscission to occur.

This was the condition of affairs in the experiment of February, 1926. Only a quarter of an inch of rain had fallen in the three months previous to the experiment, and the temperature was fairly consistently high.

These experiments, then, would indicate that moisture relations, probably both from within and without, play the predominant part in determining whether the cambium which arises round a wound shall first behave as an absciss layer, and then close the margin by cork formation, or shall proceed directly to form a protective layer of cork to cut off the injured part, without any abscission having occurred.

It is probable, however, with leaves such as those of the stone-fruit trees, which have not the size and resistant qualities of those of the cherry-laurel, that the factors age and vitality of leaf would have to be considered also.

The Origin of the Meristem.

In considering the possible mechanism underlying this type of leaf-reaction a distinction must be drawn between meristem formation and abscission. It has been seen that meristem formation in the mesophyll round the injured cells always occurs, but that abscission, involving dissolution of the middle lamellae of a certain layer of cells, does not always occur. Abscission, however, appears to depend in part upon the same sequence of changes that initiates meristem formation, for if it does supervene, it always does so at a certain stage and in a certain position in this sequence of changes.

In only one paper dealing with a shot-hole disease is the possible mechanism of abscission considered. Higgins (9) believed, in the case of shot-holes caused by *Cylindrosporium padi* on several species of *Prunus*, that separation was brought about by the enlargement of a layer of cells at some distance from the ends of the mycelium. He suggested that this enlargement was due to increase in osmotic pressure resulting from hydrolysis of amygdalin in the leaves. This hydrolysis was supposed to be brought about by wilting, caused by the rupture of the epidermis by the acervuli of the fungus, the amygdalin in the immediate vicinity of the fungus having been assimilated during the growth of the latter.

Higgins failed to notice most of the changes proceeding at the absciss line, of which the swelling up of the cells is almost the last. He also does

not compare the process with abscission in other plants which possess no amygdalin, the similarity between the two processes scarcely warranting the introduction of amygdalin into the question. His theory, moreover, would not explain the origin of the absciss layer in an exactly similar situation round spots caused by physical or chemical agents. It therefore seems unlikely that any amygdalin-hydrolysis theory will be of use in explaining the mechanism of abscission.

There is one other theory which may possibly afford an explanation of at least part of this process of leaf reaction, and that is Priestley's theory (14) of the origin of a cambium across a gradient of hydrogen-ion concentration. Indeed, the generalized nature of Priestley's theory would demand that all cases of the *de novo* origin of a meristem should be examined in its light. Following this theory, if in the present case infection led to the formation in or adjacent to the killed area of a reaction on the acid side of the iso-electric point of the leaf protein, then a circular cambium should arise at a short distance all round the infected spot, and this, as we have seen, is what actually occurs.

Experiments have been made along two lines to investigate the changes of reaction which occur near the injured tissue, therefore, and though pressure of other work has prevented their being carried to a definite conclusion, the results obtained to date may be here set down. In both cases cherry-laurel leaves were used as affording material showing the reaction on the largest scale.

The first series was designed to ascertain whether the position of the absciss line could be altered by treatment with acids and alkalis. It was argued that if the meristem arose at a definite point (the iso-electric point of the leaf protein) across a gradient of hydrogen-ion concentration, if this gradient could be increased or decreased there might be a shifting of the position of the meristem farther into the leaf-tissue or the reverse. Blackman and Matthaei (4) had already noted that up to a certain extent the wider the margin of killed cells the farther away from the injured tissue the absciss line arose. Comparisons in the present tests could only be made, therefore, when the different treatments killed an equal amount of tissue. The acid and alkali treatments were compared in each case with a simple burning treatment in which different amounts of tissue were killed by short pressure of a hot iron.

The results obtained will not be presented in detail since all the experimental difficulties have not yet been overcome. At first the wounded leaves were immersed in 2N HCl or 2N NaOH for a period of two minutes, then washed and stood in beakers with a little water in the bottom and with a watch-glass over the top, together with the burnt controls. It was found that there was sometimes considerable individual variation in the manner of reaction of different leaves, probably owing to age and other

factors, and later work was performed with all three treatments on the same leaf. It was found, when the three treatments were compared, that although an equal amount of tissue had been killed in each case, the absciss line did not arise at the same distance from the different injuries. It arose considerably farther away from the injury when the wounds had been treated with acid (Pl. XVIII, Figs. 14–16), and nearer to the injury when they had been treated with alkali, as compared with its position round the burnt tissue used as control. The treatment with alkali occasionally gave an irregular result, the absciss line arising farther from the injury than did that round the burnt tissue. Alkali treatment, moreover, always had a retarding effect upon the speed of the reaction.

In the second series, an endeavour was made to determine directly the hydrogen-ion concentration of the tissue on each side of the absciss line or meristem. Colorimetric methods are poor for leaves such as those of cherry-laurel which have little sap, practically the only way of proceeding being that of Atkins, who crushed up the leaf-tissue in a drop of distilled water on a white tile and added the indicator directly. Little more could be determined from this method than that the reaction was in the neighbourhood of pH 5.8; it was found impossible to say whether the tissue cut off by the absciss layer was any different in reaction or not.

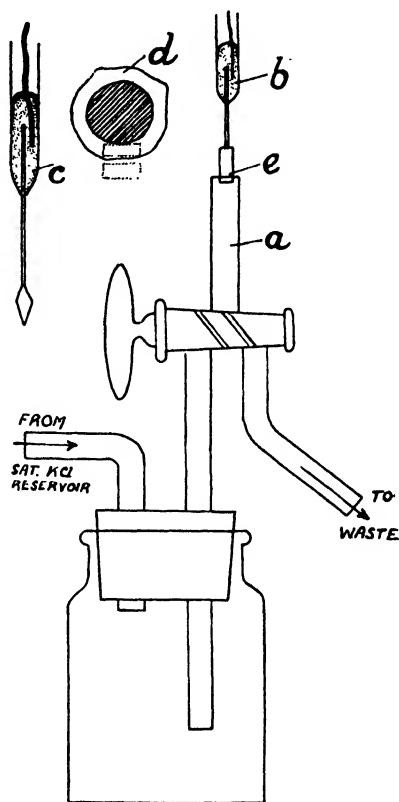
A more delicate and, it is believed, more reliable method was then adopted, namely, an electrometric determination with the quinhydrone electrode (3). It is probable that other workers have also used this method for the determination of hydrogen-ion concentration in plants, but no account of such work has been seen by the author, so that a very brief description of those parts of the apparatus which were modified for the work with plants may be given. The electrode was made from a piece of platinum wire, the end of which was flattened with a blow from a hammer and cut into the shape of a narrow diamond (Text-fig. 18, *c*). The advantage of this was that it could be inserted into a piece of leaf-tissue less than 2 mm. wide, thus avoiding all but the very smallest veins. The electrode was always heated to redness at the beginning of every set of determinations, and washed and kept in distilled water between each determination. Pieces of leaf-tissue about 2 by 5 mm. were used, a fresh lot of saturated potassium chloride being run into the tube (Text-fig. 18, *a*) from the reservoir for each determination. A double-edged scalpel was used to make a bed in the leaf-tissue for the electrode, which was just removed from the distilled water, touched into quinhydrone, and inserted into this prepared hole. The electrode was strong enough to be pushed a little farther into the tissue also. The other contact was made by just dipping the lower end of the leaf-tissue into the saturated potassium chloride (Text-fig. 18, *e*).

A number of preliminary tests were made with this apparatus on such tissues as potato, and apple and other fruit; these agreed well with published

results of colorimetric determinations on expressed sap or crushed tissue of these plants. In fact, it was found that a hydrogen-ion concentration map of a potato tuber could readily be made out, the reaction becoming slightly more acid as one proceeded outwards from the centre, the vascular circle and the tip being most acid.

The conductivity of cherry-laurel leaf is not nearly so good as that of more juicy tissue, so that the sensitivity of the apparatus when this was used was considerably less, but readings could always be made to an accuracy greater than pH 0.1. The results need not be presented in detail, for they were consistently uniform. Different leaves varied in their reaction from pH 5.6 to pH 6.0, the majority being pH 5.7 to pH 5.8. Usually the healthy lamina was fairly uniform in reaction at all parts, but sometimes a variation up to pH 0.3 was recorded for different parts. In the tests for reaction on each side of the absciss line the samples were taken as closely adjacent as possible (Text-fig. 18, *d*). In some cases it was found impossible to detect any difference in the reaction of the two samples, but usually the tissue being abscissed was very slightly more alkaline, seldom more than pH 0.1 however. In the case of the wounds treated with acid the reaction was usually reversed, the abscission tissue being very slightly more acid than the normal leaf-surface.

These results lend no support to the theory of shot-hole formation which it was set out to test. Evidently the reaction of the tissue cut off by the meristem is not so acid as to give rise to a gradient of hydrogen-ion concentration which would include an iso-electric point within the usual range for plant proteins. It is just possible that in the case of *Prunus* leaves the iso-electric point of the main protein lies very close to the normal reaction of the leaf. The peculiar way in which leaves kept for some weeks in a moist jar will



TEXT-FIG. 18. Hydrogen-ion determination in the tissue on each side of the absciss line by the quinhydrone electrode. *a*, tube filled with fresh potassium chloride solution after each determination; *b*, electrode embedded in small piece of leaf-tissue which just makes contact with KCl solution; *c*, detail of electrode, mercury in tube; *d*, method of taking samples of tissue just within and without the absciss line arising round a burnt spot on cherry-laurel leaf; *e*, sample of leaf-tissue just dipping into saturated potassium chloride at the lower end.

absciss parts of their leaf-surface in the most irregular manner is suggestive in this connexion. If this were not the explanation, it would be necessary to seek for the development of an acid reaction in a much more localized area, such as near the lignin zone described above. Although wood cells are normally fairly acid, it could not be established (e.g. by soaking sections in methyl red) that the narrow lignified zone arising in the mesophyll was also acid in reaction. Nor could any development of fats or fatty acids be demonstrated with Sudan III or osmic acid before the meristem had already arisen and the epidermis been broken. The mechanism of the origin of the meristem seems to differ considerably in detail, therefore, from that investigated by Priestley and Woffenden (15) for the case of cut potato. On account of this and also of the way in which the position of the meristem can be altered to some extent at will, the problem of its origin in cherry-laurel presents an attractive field for further inquiry.

III. SUMMARY.

1. The relative merits are discussed of the names *Clasterosporium carpophilum*, (Lév.) Aderh., and *Coryneum Beyerinckii*, Oud., both names being widely used for the same fungus, which cause a 'shot-hole' disease of the leaves of many stone-fruit trees, as well as a fruit-scab, twig-blight, &c.

Successive abstriction of a number of spores from one conidiophore, giving the latter a geniculate shape, the formation of sectorial 'mutants' in culture, and the preponderance of single conidiophores in culture instead of the aggregations characteristic of Melanconiaceous fungi, are characters described herein which undoubtedly indicate a close affinity with *Helminthosporium*. Other reasons also are given for the greater Hyphomycetous than Melanconiaceous affinity of the fungus, from which it is concluded that the name *Clasterosporium carpophilum*, (Lév.) Aderh., is the more correct.

2. The germination of the spores is described, and it is shown that the germ-tubes are surrounded by a mucilaginous sheath which fixes them to the substratum, are negatively heliotropic, and sometimes exhibit a chemotropic response in the presence of host-tissue.

3. Infection of almond leaves is shown to occur by penetration of the cuticle, which is in direct conflict with the findings of Parker in a recent paper, working with apricot and cherry leaves. The hyphae grow in the substance of, or pass directly through, cellulose walls which swell up when in the vicinity of the fungus mycelium; growth of the fungus within the leaf is described as slow and sparing, the host-cells dying in advance of the fungus hyphae.

4. The reaction of almond leaves to infection by the fungus is described. This involves the swelling up of a zone of cells in the healthy mesophyll at a little distance from the killed tissue, leading to the occlusion of inter-

cellular spaces and to a naked-eye appearance of a thin waterlogged line when the process is active. This change is accompanied by the lignification of a few layers of cells on the inside of this zone; the disappearance of chloroplasts, increase of protoplasm, and enlargement of nuclei in the mesophyll cells immediately without, which thus assume meristematic characters; and frequently a local accumulation of starch.

5. At this stage, if the moisture supply is abundant and the leaf comparatively young and active, abscission occurs by the breaking of the epidermis on both surfaces and the dissolution of the middle lamellae between the two layers of cells immediately without the lignified zone; the infected disc of tissue then drops away. It is suggested that the lignified zone aids materially in the breaking of the epidermis, by acting as a resistant barrier against which the pressure of the developmental changes proceeding without may exert a force which ultimately snaps the cuticle by tension. Cell divisions have usually occurred in the meristem before complete abscission, and the cells formed acquire suberized, and some also lignified walls, so that the margin of the shot-hole is now completely protected by a layer of wound-cork.

6. If the leaf is old or moisture relations unfavourable, abscission may not occur, in which case the meristem cells become suberized, thus cutting off the infected tissue by a barrier of wound-cork, which also serves to check further extension of the fungus into the leaf. In this case the tissue soon dies right up to the wound-cork barrier, and whether it drops out or not, leaving a shot-hole, depends solely upon whether shrinkage occurs or whether there is slight subsequent growth in area of the leaf. In either case, the dropping away is purely a physical process. The dropping out of infected tissue without any cell development on the part of the host, as described by several other writers, was not seen in the present investigation, and the accuracy of some of these accounts is questioned.

7. The influence of moisture as a determining factor in whether abscission occurs or not is dealt with by some experimental work on cherry-laurel leaves, which exhibit a similar type of abscission of injuries.

8. The possible mechanism of meristem formation round the infected tissue is discussed. Priestley's theory of the origin of a cambium across a gradient of hydrogen-ion concentration offers an attractive explanation of the process, but some experimental work to detect a difference of pH on the two sides of the meristem (using the quinhydrone electrode) gave no positive results.

The above investigation was commenced in 1922 as a subject of research for the John L. Young Scholarship in the University of Adelaide. It was suggested by Professor T. G. B. Osborn, and his continued help has been of the greatest value to the author. The cutting of the sections deal-

ing with fungus penetration, and the photomicrographs of these, were done while the author was kindly allowed the use of the Cryptogamic Botanical Laboratories of the University of Manchester, and the help of Professor W. H. Lang and Dr. Wilfrid Robinson is acknowledged with gratitude. The quinhydrone electrode work was done in the laboratory of Professor J. A. Prescott, of the Waite Agricultural Research Institute in the University of Adelaide, and his assistance in this is also gratefully acknowledged. For some of the literature required the writer is indebted to the Director of the Imperial Bureau of Mycology, Dr. E. J. Butler, and for herbarium specimens of the fungus to Mr. C. C. Brittlebank, of the Department of Agriculture of Victoria, and Dr. J. R. Weir, in charge of Pathological Collections in the Bureau of Plant Industry, United States Department of Agriculture. Dr. E. J. Butler has also kindly consented to see this paper through the press.

The work has been interrupted for several long periods, and pressure of other work precludes its being carried farther forward in the near future; it has, therefore, been decided to publish what has already been done, though the investigation of the details of abscission especially has to be left in a state far from complete.

The writer would be very glad to receive specimens or cultures of any species of *Clasterosporium* or *Coryneum*, with a view to further comparison of these genera.

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EXPLANATION OF PLATES XVII and XVIII.

Illustrating Mr. G. Samuel's paper on the Shot-Hole Disease caused by *Clasterosporium carpophilum*.

PLATE XVII.

Zeiss-Abbe camera lucida drawings of stained microtome sections.

Figs. 1, 2. Penetration of the cuticle of almond leaves by the germ-tubes from germinating spores of *Clasterosporium carpophilum*. The swelling up of the cellulose walls is probably more marked than occurs in natural infection, for the leaves were kept in a moist culture dish. $\times 1,100$ and 900, respectively.

Figs. 3, 4. Cross-sections of almond leaves, showing the absciss line arising round a *Clasterosporium* infection. The infected tissue is towards the left. $\times 525$.

PLATE XVIII.

Figs. 5, 6. Photomicrographs of microtome sections of artificially infected almond leaves, showing direct penetration of the cuticle by the germ-tubes of the fungus, and the swelling up of the cellulose walls of the host. See also Plate XVII, Figs. 1, 2.

Fig. 7. Photomicrograph of a stained microtome section of the absciss line arising round a *Clasterosporium* infection on almond leaf. See also Plate XVII, Fig. 3.

Fig. 8. Photomicrograph of a very small scab on young apricot fruit due to infection by *Clasterosporium carpophilum*.

Fig. 9. Photomicrograph of small scab on almond twig due to infection by *Clasterosporium carpophilum*.

Fig. 10. Photograph of almond leaves (different varieties) showing shot-hole infections due to *Clasterosporium carpophilum*. In one the infections have been abscised, whereas in the other they are isolated by a wound-cork barrier as in Text-fig. 11.

Fig. 11. Typical 'shot-hole' effect due to abscission of *Clasterosporium carpophilum* infections on moderately young almond leaves.

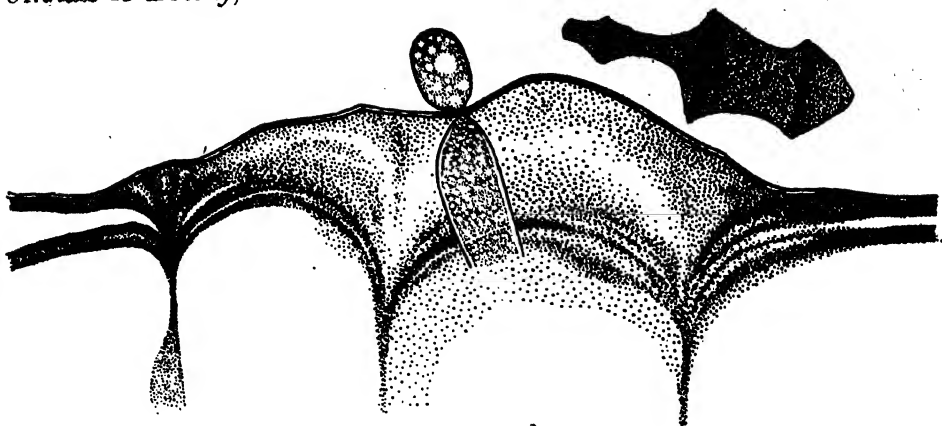
404 Samuel.—On the Shot-hole Disease and 'Shot-hole' Effect.

Fig. 12. *Clasterosporium* infections on almond twigs. The mycelium over-winters in these scabs and produces conidia in spring which probably serve to initiate the infections of the new season.

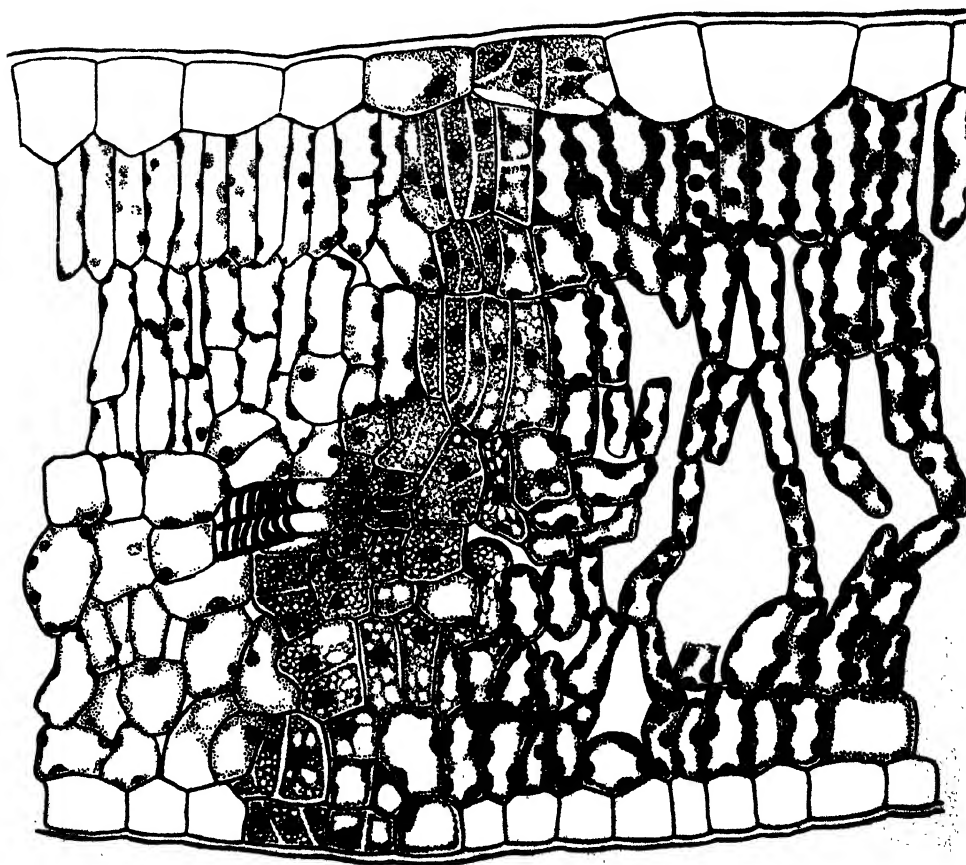
Fig. 13. Single-spore culture of *Clasterosporium carpophilum* on Quaker oats agar, showing origin of a 'mutant' sterile sector.

Fig. 14. Cherry-laurel leaf wounded and treated with dilute HCl for two minutes, showing absciss line arising a considerable distance away from the killed tissue.

Figs. 15, 16. Cherry-laurel leaves showing variation in position of absciss line when wounds are treated with acid or alkali. Top right circle burnt with a circular hot iron and central tissue immediately removed with a cork-borer; lower right circle and line treated for two minutes with 2N NaOH; left circle and line treated for two minutes with 2N HCl.

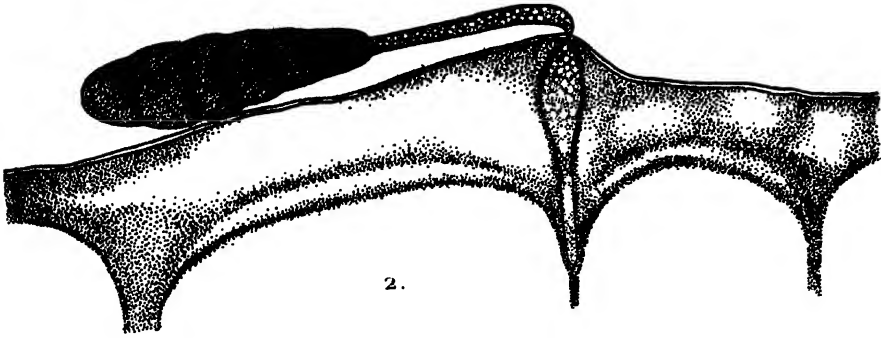


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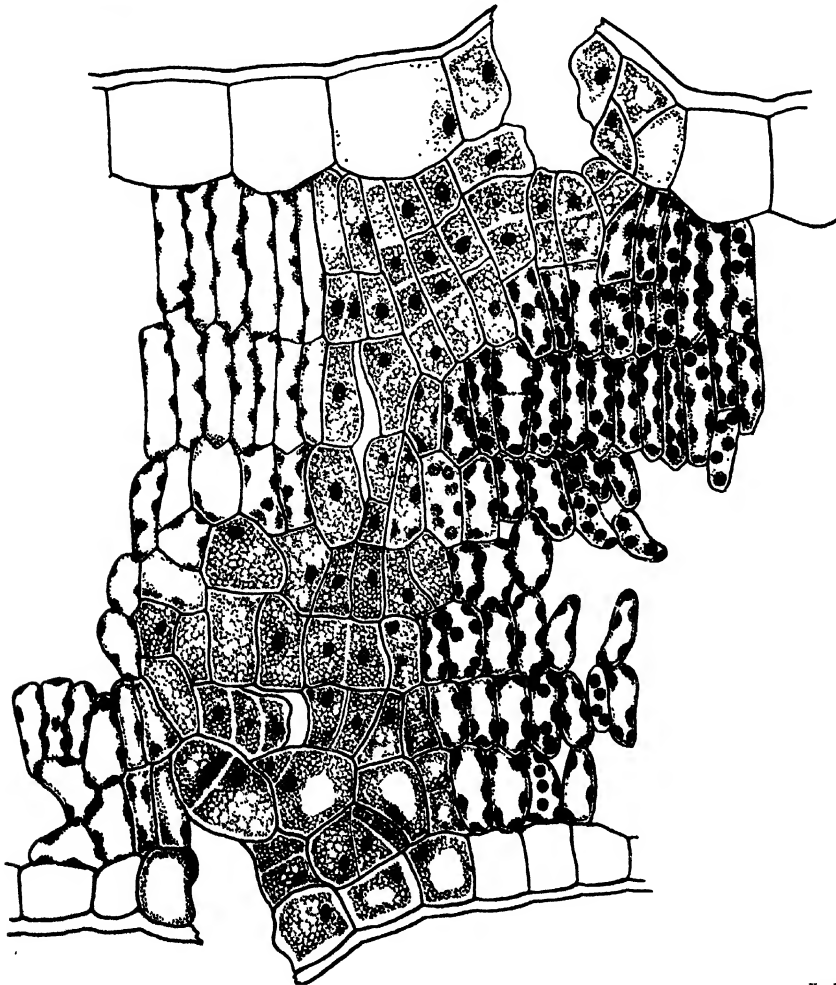


3.

SAMUEL-CLASTEROSPORIUM.



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Huth coll



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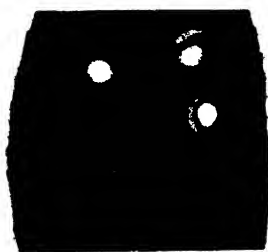
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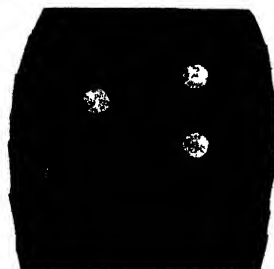


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NOTES.

THE STORAGE CARBOHYDRATE OF THE LEAF OF GOSSYPIUM.—

As there appears to be no reference to the storage product of photosynthesis of *Gossypium* in the literature of the cotton plant, the following note may be of interest.

When cotton leaves are tested for starch with iodine solution by the ordinary methods, no deep blue colour is produced: a totally distinct light brown or purple-brown colour appears in the palisade and spongy mesophyll tissues.

From the fact that oil, as revealed by osmic acid, is constantly present, not only in the mesophyll tissues of the leaf, but also wherever green tissues are developed, e. g. the stem and the boll, it might seem possible that this was the temporary storage product of photosynthesis. This oil, however, does not disappear from leaves kept in the dark. Further, there is scarcely any diurnal variation in quantity, samples of leaves collected in the evening and morning yielding 5.4 per cent. and 6.0 per cent. of the dry weight of substances soluble in carbon tetrachloride, while the decrease in dry weight amounted to 17.7 per cent. Oil cannot, therefore, be the storage product concerned.

Providing certain precautions are taken, it is possible to demonstrate the presence of starch in the cotton leaf. After the leaf has been killed, it must be decolorized very thoroughly in alcohol, and the iodine solution used for the test must be very dilute (0.5 gm. of iodine per litre). This solution is allowed to act for 2-3 hours, when the blue colour of the starch compound becomes obvious. If the leaf is treated with a solution of caustic potash, neutralized, and washed thoroughly before the iodine solution is allowed to act, the colour is much more obvious, and by this method it is possible to obtain excellent starch prints. It is therefore concluded that starch is the temporary storage product of photosynthesis in the cotton plant.

It is interesting to note that the case of *Musa* is very similar, Briosi (1873)¹ having maintained that in many of the Musaceae oil was the assimilation product, since there appeared to be no starch present, while Holle (1877)² denied that this was the case, though apparently he also found no starch. Finally, Godlewski (1877)³ was able to demonstrate the presence of starch in the leaf, and concluded that this was the storage product of photosynthesis. Godlewski's results were confirmed in the case of banana, and starch prints were obtained by the method described above.

¹ Briosi, G.: Ueber normale Bildung von fettartiger Substanz im Chlorophyll. Bot. Zeit., xxxi, 34, pp. 529-33; 35, pp. 545-50, 1873.

² Holle, H. G.: Ueber die Assimilationstätigkeit von *Stralitsia reginae*. Flora, lx, pp. 113-20, 154-68, 184-92, 1877.

³ Godlewski, Emil: Ist das Assimilationsprodukt der Musaceen Öl oder Stärke? Flora, lx, pp. 215-20, 1877.

The writer wishes to acknowledge the help of Mr. C. H. Wright, of this Department, who kindly carried out dry weight and oil determinations of leaf samples, and he is greatly indebted to Dr. E. J. Butler, Director of the Imperial Bureau of Mycology, for pursuing inquiries and supplying abstracts of literature.

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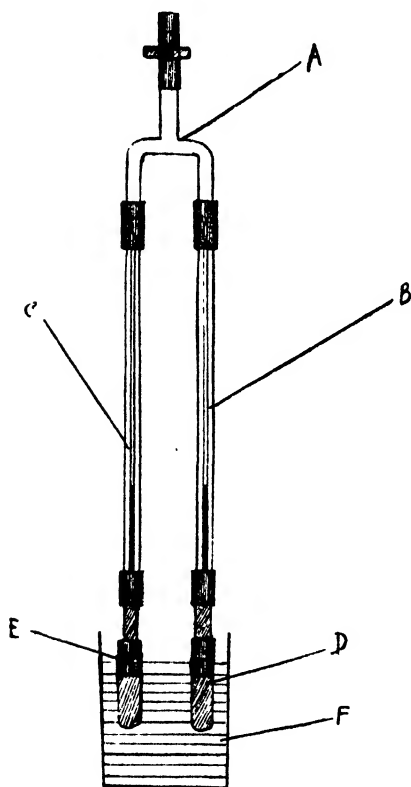
November, 1926.

AN APPARATUS FOR DEMONSTRATING EXUDATION OF WATER.

—In his extremely interesting paper on 'Osmotic Pressure, Root Pressure, and Exudation' (New Phyt., xx, p. 106), Professor V. H. Blackman has described a model (Fig. 3, p. 113) to illustrate Pfeffer's second hypothesis for explaining the exudation of water from a plant-cell. It is supposed, in this, that there is an unequal distribution of osmotic material in different parts of the cell; hence water is drawn into the cell at one point after equilibrium has been reached at the other; accordingly water is exuded, as described below. The phenomenon of exudation is particularly well known in the sporangiophore of *Pilobolus*.

It was thought that it would be of interest to devise a working model that could easily be set up, and that could be used for demonstration purposes to illustrate the above-mentioned theory. The apparatus described below is based on Blackman's model (loc. cit., Fig. 3) and fulfils these requirements.

In the accompanying figure **A** is an appropriately bent T-piece fitted at one end with a piece of pressure tubing and a clamp, and at the bent ends connected by pressure tubing to two pieces of thermometer tubing



(**b** and **c**). This part of the apparatus is filled with medicinal paraffin, the oil being drawn in, through the capillaries, by means of a vacuum pump, and the clamp then being screwed tight. **d** and **e** are two collodion osmometers, prepared according to the method described by Brown (this Journal, xxxvi, p. 433), and both fitted with short pieces of glass and pressure tubing. **e** is filled with a strong solution of cane sugar, **d** with a very dilute solution. Both solutions are coloured with ordinary writing ink. **e** and **d** are now attached to the capillary tubes **c** and **b**, the slight pressure caused by the attachment of the second sac being absorbed by the elasticity of the rubber connexions and the osmometers.

D and E are now immersed in a vessel of water (F) and left for some time (all night if required).

The method of working may be described as follows, using Blackman's words almost verbatim: On the entry of water through E and D, pressure will develop in the system, and when the pressure reaches that of the osmotic pressure of the solution above D, absorption through D will stop, since, owing to the pressure, the solution on the one side of the membrane will be in equilibrium with the water on the other side. At E, however, there will be no equilibrium at the two faces of the membrane, owing to the higher concentration of the solution, and water will continue to enter. As a result the pressure in the tube will go on increasing, and there will no longer be equilibrium at D, but water will be forced out, and the solution in that sac will become more concentrated.

That this takes place is shown by the level of the solution in C rising and that in B falling; this result was checked by a number of observations with different sacs. In one experiment the same result as regards change of level of the solutions was observed when D was surrounded by an atmosphere saturated with water vapour. In this case, drops of water appeared on the outside of the sac, increasing the resemblance to the phenomenon as seen in *Pilobolus*.

The liquid paraffin prevents the two solutions in E and D from ultimately mixing, and may be taken to represent the energy barrier present within the living cell, postulated in Pfeffer and Blackman's theory. It is essential that the solution in D should be dilute, since it determines the pressure within the apparatus, and the sacs are ruptured by high pressures, though their strength is rather surprising. In the experiments performed here, E contained a solution of 1.75 M., while D was filled with one of approximately 0.035 M. An obvious change in level occurred in about 90 min.

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Studies in the Cytology of the Anacrogynae.

IV. Fertilization in *Pellia Fabbroniana*.

BY

AMOS M. SHOWALTER.

With Plates XIX-XXI.

THE experimental part of this study, together with the fixation and embedding of material, was done during my residence in Belgium as an Exchange Fellow of the C. R. B. Educational Foundation. The embedded material has been sectioned and studied under tenure of a National Research Fellowship in the Biological Sciences.

Plants of *Pellia Fabbroniana* were collected in the Forêt de Soignes along the ravine which emerges from the forest at Rouge Cloître. This species was found generally nearer the water-level and in situations more humid than was *Pellia epiphylla*, which latter was abundant over a larger area of the forest. The two species were found occasionally in close proximity, but they are easily distinguished, especially when fruiting, and no evidence of hybridization was found. Both species produce sporophytes in abundance.

P. Fabbroniana grew well and produced sporophytes in greenhouse cultures, but *P. epiphylla* was almost invariably killed by fungal parasites within a few weeks after being transferred to the greenhouse. Cultures of *P. Fabbroniana*, male and female plants intermingled, were started in the autumn of 1922 in the Bryophyte greenhouse of the Jardin Botanique de l'État at Brussels. In January 1924 female plants were transplanted to separate cultures in seed pans about 25 cm. square. These plants proliferated freely, and in June formed compact cushions with thousands of archegonial branches. Antherozoids were obtained more readily and in greater abundance from male plants collected in the field than from plants in culture.

The fixation of *Pellia* is attended with difficulties. The cells of the surface of the thallus and of the involucre surrounding each group of archegonia contain many 'elaiophlasts' with an abundance of fatty substances. Osmic acid acts very violently on these cells, and is deposited in them, but,

unless used in excess, does not penetrate to the inner cells of the archegonial branch.

The solution used for fixing *Riccardia* and *Fossombronia* (formula in second paper of this series) gave generally good fixation of young archegonia, of the egg, and of zygotes up to the age of four days, but the thallus is in all cases very unevenly fixed. The fixation of older zygotes, and especially of multicellular embryos, is unsatisfactory. This may possibly be due to the increased thickness of the involucre and of the developing 'calyptra' which surround the zygotes.

This study of fertilization is based mainly on two series of fixations following inseminations in 1924, on June 26 and 27 respectively. The maximum temperature in the greenhouse on each of these two days was about 26° C. In the former instance one side (about half) of one culture was submerged in water, to which was then added water containing free-swimming antherozoids. In the second instance a whole culture was submerged and inseminated.

For a third series of fixations the same culture, of which one side had been inseminated on June 26, was inseminated on July 4. Thus a considerable number of archegonial branches bearing eight-day-old embryos were re-inseminated, and in several instances early stages of fertilization were found in archegonia on the same branches with the older embryos. At this date and following, the corresponding daily temperatures in the greenhouses were approximately 5° lower than on June 26 and 27. The results from this third series of fixations show considerable divergence from those of the two former series and will be discussed later.

For fixations earlier than five minutes after insemination, female plants were removed from the soil and placed in water for fifteen minutes. Part of these were then placed in a phial, and water with actively swimming antherozoids was added. After one minute this water was replaced with fixing fluid. Another lot inseminated in the same manner was fixed after 2 minutes 5 seconds and a third lot after 3½ minutes.

Serial sections were cut at from 5 to 12 μ thickness and stained with the triple stain. Iron-alum-haematoxylin also was used for comparison, but in general was found to be less satisfactory than the former stain. Sections unbleached and unstained were studied also. The neck of the archegonium of *P. Fabbriana* is longer and the neck canal much narrower than in the archegonium of *Riccardia* (Showalter (14)). The egg is larger than that of *Riccardia*, but varies considerably in size and shape. It is usually ovoid and fills almost completely the venter of the archegonium (Pl. XIX, Figs. 1, 2). In some cases it is spherical or even flattened on the side towards the neck of the archegonium; in other cases, at the opposite extreme, its length is more than twice as great as its transverse diameter.

The cytoplasm is granular and contains many small globular bodies which are blackened by the osmic acid and which are probably analogous to those found so abundantly in the spores of this species (Showalter (12)), and to some extent in the cells of the thallus. These bodies are usually not perceptible after bleaching and staining with the triple stain, but are frequently distinguishable in preparations stained with haematoxylin. The surface of the egg is smooth and is limited by a delicate plasma membrane. The nucleus lies at or near the centre of the egg, and is in the 'resting' condition with the chromatic reticulum fairly evenly distributed throughout the nuclear cavity. It contains one nucleole (very rarely two, e.g. Pl. XIX, Fig. 3).

Material fixed one minute after insemination shows no case in which the antherozoid had reached the egg. That fixed 2 minutes 5 seconds after insemination shows eight cases, in each of which one or more antherozoids had applied themselves to the surface of the egg and had undergone a perceptible reduction in thickness similar to that described in *Riccardia* (14); one case in which an antherozoid had reached the venter of the archegonium but seems not to be applied closely to the surface of the egg or altered in thickness; and seventeen cases in each of which an apparently functional egg had not yet been reached by any antherozoid. In the material fixed $3\frac{1}{2}$ minutes after insemination most of the eggs show reduced antherozoids, or male nuclei, applied to them, but the number was not counted. Only rarely are antherozoids found in the necks of fixed archegonia.

In material fixed five minutes after insemination (of the whole culture) nearly all eggs show one or more antherozoids applied to the surface of each of them and reduced in thickness (Pl. XIX, Fig. 1). The antherozoid of *Pellia Fabbroniana* is markedly smaller than that of *Riccardia pinguis* (18), and the reduction in thickness when it becomes applied to the surface of the egg is usually somewhat less obvious than in *Riccardia*. The smaller size of the antherozoid and the narrowness of the space between the egg and the wall of the archegonium make it more difficult to determine the exact position of the male nucleus with relation to the plasma membrane of the egg. It is in the most favourable cases very difficult, and in the majority of cases utterly impossible, to determine with certainty whether the male nucleus is just without or just within the plasma membrane of the egg in material fixed during the first half-hour after insemination. These stages in this species show in most cases more than one male nucleus applied to, or entering, the egg, whereas in *Riccardia pinguis* only rarely is more than one so applied, although in this latter species scores of antherozoids may be packed in the neck and venter of the archegonium.

Because of the difficulties just mentioned, I am unable to determine the time required for the male nucleus to pass through the plasma membrane of the egg. In material fixed forty minutes or longer after insemina-

tion it is in some cases evident that one (or more) of the male nuclei is on the inside (Pl. XIX, Fig. 2, upper right). I am unable to determine with certainty whether the two male nuclei shown below in Fig. 2 are on the inside or the outside of the membrane. Polyspermy is frequent in this species, but in most of the cases found in my material only one male nucleus has entered the egg; the others remain on the surface.

Occasionally there is found in the cytoplasm of the egg or on its surface an anomalous structure which I am unable to identify. Its appearance is suggestive of an empty membranaceous sheath, such as might have formed the peripheral layer of the antherozoid, but it is found too infrequently to justify a definite conclusion that it is such. In the case represented by Pl. XIX, Fig. 3, it is quite obvious that the structure in question lies in the cytoplasm of the egg. In other instances it seems to be adhering to the surface of the egg.

The membrane of the egg undergoes no visible change during or immediately following the entrance of the male nucleus. This is in harmony with the conditions in *Sphaerocarpos* (10), *Riccardia* (14), and *Fossombronia* (15).

The male nucleus passes slowly inward from the surface of the egg and becomes coiled in various forms, most frequently approaching a spiral, but at first without any perceptible change in length or thickness (Pl. XIX, Figs. 4, 5).

After about ten hours the male nucleus undergoes an abrupt change of form, becoming short and thick (Pl. XIX, Figs. 7-11). In fixations 10 hours 40 minutes and 12 hours respectively after insemination most of the male nuclei in the eggs have undergone this change, but some are not perceptibly changed from the condition represented by Pl. XIX, Figs. 4 and 5. Intermediate stages such as that shown in Fig. 6 are found only rarely.

Very soon after this change in the form of the male nucleus one or more vacuoles appear in the cytoplasm immediately adjacent to it (Pl. XIX, Figs. 7-10). These vacuoles increase in number and size, and coalesce as the septa between them become more and more attenuate (Pl. XIX, Figs. 11-13). A remnant of these septa remains, however, in the form of a few delicate filaments which connect the male nucleus with the membrane of the fusion vacuole, in which the male nucleus now appears to be suspended (Pl. XIX, Figs. 14-16). The formation of vacuoles immediately adjacent to the male nucleus following its change of form suggests the possibility of a localized metabolic activity resulting from the interaction of the male nucleus with the surrounding cytoplasm of the egg.

The male nucleus becomes optically non-homogeneous and its outline becomes uneven (Pl. XIX, Figs. 12-16). It is now a mass of chromatin in a large vacuole, and the only visible membrane surrounding it is the mem-

brane of this vacuole, which is obviously of cytoplasmic origin. From this stage on to the fusion of the sexual nuclei the outer limit of the male nucleus is not clearly defined. The absence of any visible membrane about the male nucleus in these stages (Pls. XIX and XX, Figs. 12-23) suggests that the liquid, or continuous, phase of the colloidal system composing the male nucleus may be chemically similar to, and physically continuous with, the contents of the vacuole.

During these changes the male nucleus lies near the female, which latter becomes more or less lobed and indented, usually with a concavity on the side towards the male nucleus (Pls. XIX and XX, Figs. 15-19). The paternal chromatin becomes more disperse, but does not fill the volume of the vacuole in which it lies, and one or more nucleoles appear in it (Pls. XIX and XX, Figs. 15-23). The granular cytoplasm between the two sexual nuclei recedes, leaving the mass of paternal chromatin almost or quite in contact with the membrane of the female nucleus (Pl. XX, Figs. 21-3). This membrane disappears in the region of contact with the paternal chromatin and a new membrane appears over the paternal chromatin, separating it from the cytoplasm (Pl. XX, Figs. 24, 25). This new membrane is continuous with the membrane of the female nucleus, and the two parental masses of chromatin are now in contact and are enclosed in a common membrane. In the case represented by Pl. XX, Fig. 24 a plane of demarcation between the two masses of chromatin is still perceptible in a part (at the left) of the area of contact, while in the rest of the area the two masses are in contact with no visible plane of demarcation between them. In the case represented by Pl. XX, Fig. 25 there is no visible plane of demarcation between the two masses of chromatin, but the membrane over the paternal chromatin is very delicate. I am not absolutely certain that it is continuous over quite all of the area in contact with the vacuole. In some, if not in all, cases a portion of the vacuole previously surrounding the male nucleus is left outside the fusion nucleus (Pl. XX, Figs. 24-6). The reappearance of a membrane over the paternal chromatin immediately after the dissolution of the nuclear membrane between the two parental masses of chromatin (Pl. XX, Figs. 24, 25) suggests that the nucleolymph of the female nucleus may diffuse through the mass of paternal chromatin, and that the new membrane may be formed when this nucleolymph meets the cytoplasm.

The union of the two sexual nuclei occurs usually at from twenty-four to thirty hours after insemination (in the material fixed after inseminations on June 26 and 27). The paternal chromatin quickly assumes the condition characteristic of the maternal, and the dual nature of the fusion nucleus remains distinctly evident for a short time only (Pl. XX, Figs. 26-31) except for the presence of two nucleoles.

The zygote has been growing steadily since the first entrance of the male nucleus and its cytoplasm has become gradually less dense (Pls. XX

and XXI, Figs. 17, 32). By the time the nuclear fusion is complete the cytoplasm is distinctly vacuolate (Pl. XXI, Fig. 32).

The first segmentation of the zygote occurs usually on the sixth or seventh day, but I have only a small number of stages of this mitosis, and these show imperfect fixation. In so far as these cases can be trusted, they show essentially the same conditions as obtain in *Fossombronia* (15).

Most of the cases of polyspermy were found in the material of the series of fixations after insemination on July 4. Whether these were due entirely to an excess of antherozoids or in part to the lower temperature (or perhaps to some other cause) I am unable to say. This series of fixations was extended only to 80½ hours, and the ultimate fate of the polyspermic zygotes is in doubt.

Most frequently the two (or more) male nuclei which have entered the egg proceed concurrently to undergo the changes preparatory to fusion with the female nucleus, and approach this latter, but I am not sure that any fusion occurs in any such cases (Pl. XXI, Figs. 34, 35). Sometimes two male nuclei lie close together and occupy in common a single vacuole. There is some evidence suggestive of an occasional triple fusion, but it is not conclusive.

Less frequently one male nucleus undergoes the normal changes and fuses with the female nucleus, leaving the other rod-shaped male nucleus, or nuclei, in the peripheral part of the cytoplasm (Pl. XXI, Fig. 33). It seems not improbable that polyspermic zygotes of this type may survive and develop normally as in *Fossombronia* (15). Rickett (10) finds polyspermy comparatively frequent in *Sphaerocarpos*, but thinks that all such zygotes disintegrate.

In regard to polyspermy the three forms thus far studied show marked divergences. The egg of *Riccardia* (14) is very efficient in excluding supernumerary male nuclei at the membrane, but the one conclusive case in which two had passed this membrane shows both penetrating the female nucleus. The egg of *Fossombronia* admits freely at its membrane, but allows only one to unite with the female nucleus (15). The egg of *Pellia* is less efficient than that of *Riccardia* in excluding supernumerary male nuclei at its surface, but shows some capacity to control them after they are inside the cytoplasm.

The material of the third series of fixations shows great irregularity as to the time of the nuclear fusion. Stages in this fusion similar to those represented by Pl. XX, Figs. 20–8 are found in fixations at 37 hours, 43 hours, 50 hours, and 60 hours respectively after insemination.

Stages of fertilization comparable to some of these in *Pellia* have been observed in *Riccia natans* (6), *Riccia Frostii* (1), *Corsinia* (9), *Preissia* (7), *Reboulia* (17), and in *Anthoceros* (11), but in none of these plants is the whole history known. In *Sphaerocarpos* (10) and in *Riella* (8) the male

nucleus goes farther and organizes its chromosomes, which take their places with those of the female nucleus on the achromatic spindle of the first mitosis, with or without (undetermined) a fusion of the nuclei.

Centrosomes have been described in the fertilized egg of *Preissia* (7), and in the germinating 'spores' of *Pellia* (2, 3, 4, 5, 16). I have seen none in this study, but imperfect fixation of the later stages has not permitted a satisfactory study of the achromatic structures of the first mitosis.

A few female plants of *P. Fabbroniana* were inseminated with antherozoids from the unidentified form of *Pellia* of which only a single male plant has been found and of which the antherozoids are described in the first paper of this series. The female plants thus inseminated were fixed, sectioned, and studied in the usual way. They showed apparently normal eggs, but in no case was an antherozoid found to have entered the archegonium. The cultures of this male plant and its progeny (vegetative) were lost, by accident, without authoritative identification. There is, however, scarcely any doubt that it was *P. Neesiana*, male plants of which I have found more recently (1926) near Brussels and cultivated in the Bryophyte greenhouse of the Jardin Botanique.

No other attempts were made to obtain fertilization of the eggs of this species by foreign antherozoids.

SUMMARY.

1. In *Pellia Fabbroniana*, as in *Riccardia*, the antherozoid applies itself to the surface of the egg and immediately undergoes a reduction in thickness. The reduced antherozoid, or male nucleus, passes through the membrane of the egg, but the exact manner or time of this passage was not determined.

2. The slender rod-shaped male nucleus remains for about ten hours, without significant change of form, in the cytoplasm of the egg.

3. After about ten hours in the cytoplasm the male nucleus approaches the female nucleus and undergoes an abrupt change of form, becoming shorter and thicker.

4. Immediately after this change in the form of the male nucleus vacuoles appear in the cytoplasm immediately adjacent to it. These vacuoles increase in number and later in size, until they coalesce into one vacuole which surrounds the male nucleus.

5. The male nucleus soon becomes optically non-homogeneous, and its smooth outline disappears. From this stage on to the time of its union with the female the male nucleus is without a visible membrane.

6. The fusion of the sexual nuclei occurs usually at from twenty-four to thirty hours after the insemination of the female plants.

7. The first segmentation of the zygote occurs usually on the sixth or seventh day.

8. Cases of polyspermy are relatively frequent in this species. In most of these cases there is no nuclear fusion, and the zygote probably disintegrates.

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EXPLANATION OF PLATES XIX-XXI.

Illustrating Dr. Showalter's paper on Fertilization in *Pellia Fabbronia*.

All figures were drawn from permanent preparations with the aid of the camera lucida at the magnification stated, and are reproduced without reduction or enlargement.

To indicate the time elapsed after insemination the symbol ° is used for hours and ' for minutes.

PLATE XIX.

Fig. 1. Egg with two male nuclei applied to its surface, one antherozoid near the egg, but not applied or reduced in thickness : 0° 05' after insemination. $\times 2,100$.

Fig. 2. Egg with one male nucleus in cytoplasm, two others either inside or on the surface : 3° 40' after insemination. $\times 2,100$.

Fig. 3. Egg containing one male nucleus and an anomalous sheath-like structure, archegonium sectioned transversely: $0^{\circ} 50'$ after insemination. $\times 2,100$.

Fig. 4. Egg containing male nucleus: $1^{\circ} 50'$ after insemination (arrow indicates direction of long axis of archegonium). $\times 2,100$.

Fig. 5. Egg containing two male nuclei: $5^{\circ} 28'$ after insemination. $\times 2,100$.

Fig. 6. " " one male nucleus which is becoming shorter and thicker: $8^{\circ} 30'$ after insemination. $\times 2,100$.

Fig. 7. Male nucleus in cytoplasm of egg: $10^{\circ} 40'$. $\times 2,100$.

Fig. 8. " " " " " " " "

Fig. 9. " " " " " " " "

Fig. 10. " " " " " " " "

Fig. 11. " " " " " " 12° "

Fig. 12. " " " " " " $10^{\circ} 40'$ "

Fig. 13. Zygote: $10^{\circ} 40'$. $\times 2,100$.

Fig. 14. Male nucleus in cytoplasm of egg: 12° . $\times 2,100$.

Fig. 15. Male and female nuclei: 12° . $\times 2,100$.

Fig. 16. " " " " " " "

PLATE XX.

Fig. 17. Zygote: $19^{\circ} 40'$. $\times 2,100$.

Fig. 18. Male and female nuclei: $19^{\circ} 40'$. $\times 2,100$.

Fig. 19. " " " " $23^{\circ} 35'$. $\times 3,200$.

Fig. 20. " " " " $26^{\circ} 30'$. "

Fig. 21. " " " " " " "

Fig. 22. " " " " " " (haematoxylin stain).

Fig. 23. " " " " $23^{\circ} 35'$. "

Fig. 24. " " " " in act of fusing: $26^{\circ} 30'$. $\times 3,200$.

Fig. 25. Zygote nucleus just after fusion: $26^{\circ} 30'$. $\times 3,200$.

Fig. 26. " " " " " $26^{\circ} 30'$. "

Figs. 27-9. " " soon " " $26^{\circ} 30'$. "

Figs. 30-1. " " dual nature still evident: 34° . $\times 3,200$.

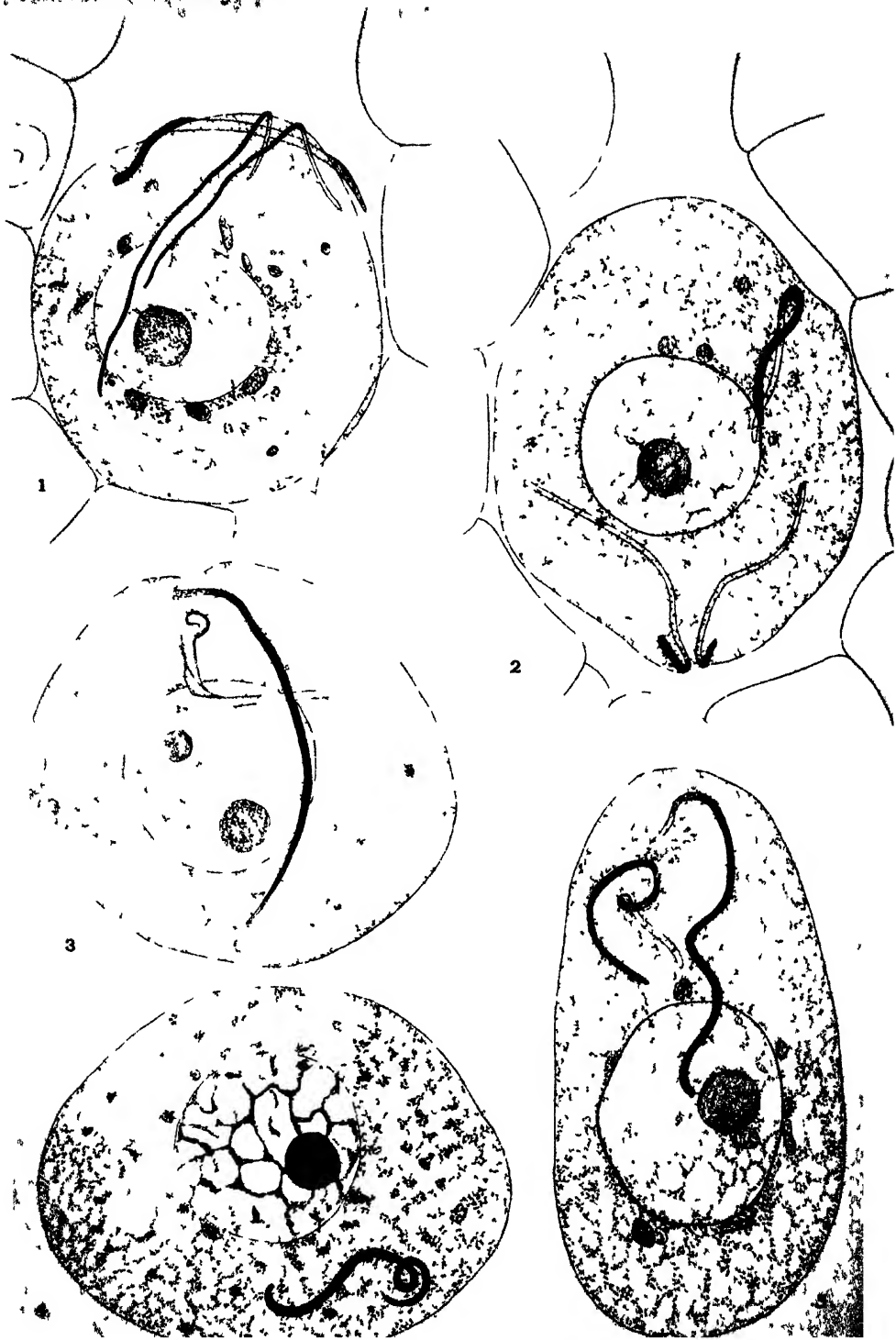
PLATE XXI.

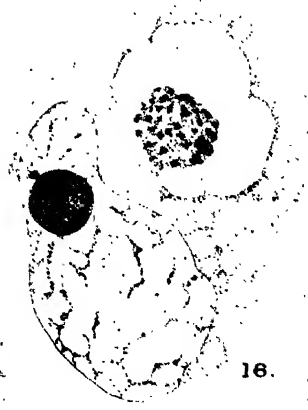
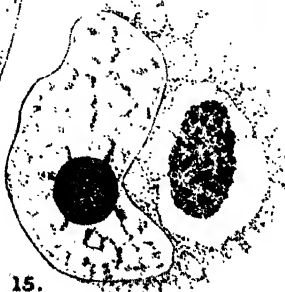
Fig. 32. Zygote: 34° . $\times 2,100$.

Fig. 33. Zygote showing fusion nucleus and one supernumerary male nucleus: 50° . $\times 1,700$.

Fig. 34. Two male nuclei in contact with female nucleus: $26^{\circ} 30'$. $\times 3,200$.

Fig. 35. Two male nuclei near female (no fusion), starch grains in plastids: $80^{\circ} 15'$. $\times 2,100$.







17



18



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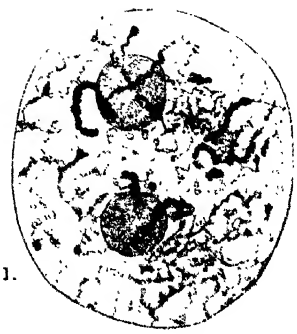
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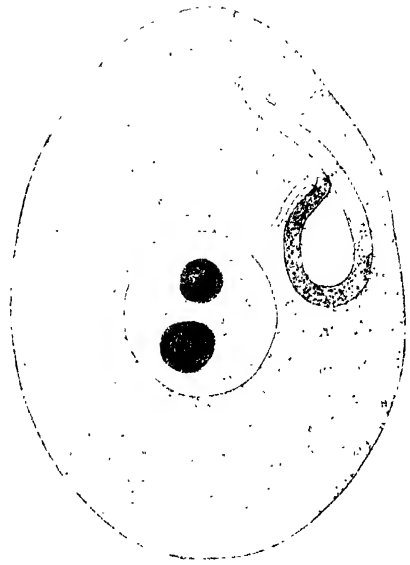
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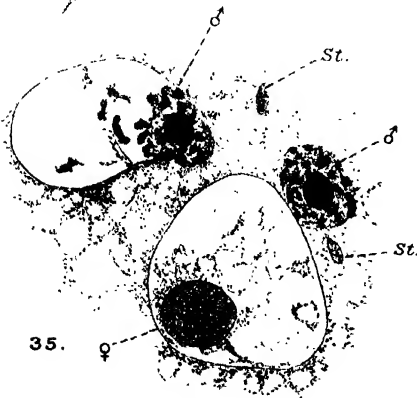
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34.

The Life-history of *Zygorhynchus Moelleri*, Vuill.¹

BY

ETHEL GREEN, M.Sc.

With ten Figures in the Text.

THE structure and development of a specimen of *Zygorhynchus Moelleri*, collected on soil in Windsor Great Park in September, 1925, have been investigated.

HISTORICAL SURVEY.

In 1886 Vuillemin (1) described a peculiar mould, which, on account of the marked inequality of its gametangia, he named *Mucor heterogamus*. Fischer (2), in 1892, suggested the need of a new genus, and Vuillemin (4), receiving from Moeller a specimen found in the soil at Eberswald and somewhat similar to *M. heterogamus*, established for both, in 1903, the new genus *Zygorhynchus* (*zugon* = yoke; *rhynchos* = beak).

The Eberswald species he named *Z. Moelleri*. It has since been recorded in almost all investigations of soil, both in Europe (10, 13) and North America. Its first recorded appearance is on bread in Harvard Laboratory, 1884. The specimen was lost, but a description of it was found to agree with Vuillemin's diagnosis.

In 1904 Blakeslee (5) published an account of the formation of the zygote by the union of unequal 'gametes' cut off from unequal 'progametes'. He studied the influence of humidity, temperature, and food material on the development of the zygospores. He said that azygospores were not infrequently formed, and apparently always from the smaller 'gamete', as they were never observed on the actively growing 'progamete'. It is interesting to note that azygospore formation has not been observed in heterothallic *Mucors*.

In discussing the sexuality of the fungus, Blakeslee stated that there was insufficient evidence to permit any definite conclusion, but he was inclined to regard the smaller as the male gamete.

Vuillemin (11), in 1908, regarded heterogamy in this case as a step towards the total disappearance of sex. He found that in *Z. heterogamus* azygospores were formed by the larger 'gamete', which was always cut off

¹ Thesis approved for the Degree of Master of Science in the University of London.

from the larger actively growing 'progamete', through which the food supply was diverted. Consequently the other 'gamete', by virtue of its position, received little food, remained small, and was tending to disappear.

Grossman (15, 23), in 1911, recorded the occurrence of *Z. Moelleri* in Michigan. He failed to observe any gametangia, but described and figured 'a complete passage way' between the copulating branches.

Moreau (16, 17), in 1911, published a preliminary account of the cytology of a species of *Zygorhynchus* which was closely akin to *Z. Moelleri* and afterwards named *Z. Bernardi*. He insisted on the use of the term 'gametangium', suggested by Dangeard (6) in 1906, and in this species distinguished two gametangia corresponding to the 'gametes' of Vuillemin and Blakeslee.

In 1912 Grüber (19) interpreted the zygosporangium as a female gamete, and the passage of the food from the curved branch into it as a fertilization process.

In the same year Moreau (20, 21, 22) pointed out the incorrectness of this interpretation, and in 1913 (25, 26) published the collective results of his cytological investigations of four species of *Zygorhynchus*.

Atkinson (18), in 1912, described the sexual apparatus of *Zygorhynchus* as being of the same type as that found in the Laboulbeniales. This view was founded on observations similar to Grüber's, and subsequently withdrawn in a letter to Blakeslee (24).

Meanwhile physiological investigations had been made along the lines originally indicated by Klebs (3) on the effect of external conditions on the development of *Zygorhynchus*.

Hagem (8), in 1907, mentioned that on almost every substratum, at every temperature, large masses of zygosporangia of *Z. Moelleri* were formed.

In 1908 Hall (9) and his colleagues at Rothamsted found that this fungus was able to obtain nitrogen by decomposing ammonium salts, and that, in so doing, it made the medium acid. Accordingly *Z. Moelleri* is one of the fungi causing acidity of the soil.

During the same year Wiśniewski (12) carried on extensive investigations on the influence of food, temperature, light, and humidity on the growth of the mycelium, and the relative production of zygosporangia and sporangia. He, too, showed that the fungus decomposes ammonium sulphate, making the medium acid and limiting its growth. Briefly, from his general results, he concluded that high temperature (about 22° C.), low concentration of food materials, and diffuse light, aid the conduction of food to the aerial hyphae, and so favour the production of zygosporangia. The reverse conditions make the conduction of food to the aerial hyphae difficult, and favour the production of sporangia.

Namysłowski (14), in 1920, investigated the effect of external conditions

on reproduction. He found that on media rich in nitrogen, and containing no carbohydrate, only sporangia were formed. This was also the case when the medium contained 30 per cent. glucose, but in his sixth conclusion he stated that culture media rich in carbohydrate favoured the production of zygosporoes.

By varying the concentration and the form in which the nitrogen was supplied, he was able to obtain cultures with neither zygosporoes nor sporangia, with both, or with only a few abnormal zygosporoes. With gelatin and a high percentage of glycerine only a few zygosporoes were formed.

His experiments showed that the ratio of the number of zygosporoes to that of sporangia varied considerably according to the culture medium and the age of the culture, yet in his fourth conclusion he said, 'Le rapport du nombre des zygosporoes à celui des sporanges est un caractère spécifique, constant, et différent chez les différentes espèces'.

More recent experiments have been concerned with investigations of the possible differentiation of the gametangia, or of branches of the fungus, into plus and minus, as in the heterothallic *Mucors*.

Blakeslee (29), in 1920, stated that his hybridization experiments with *Z. heterogamus* seemed to indicate that the gametangia may be variable with regard to sign, but his investigations were not sufficiently complete to warrant any definite conclusion.

Burgeff (30), in 1924, reported various experiments in hybridization and parasitization in heterothallic and homothallic *Mucoraceae*. As a result of hybridization experiments he distinguished certain regions of the zygosporoes of *Z. exponents* as plus, minus, and neutral, but this differentiation was not constant. The results of the parasitization experiments gave no help in the discovery of sexual differentiation.

No account has been given of zygosporoe germination in the genus. Many investigators have found sporangiosporoes to grow readily, but only Blakeslee (7), in 1906, mentioned experiments with zygosporoes, and he stated that at the time of writing zygosporoes of *Z. Moelleri*, sown in van Tieghem cells, had not germinated. Apparently they had been left for about four or five weeks.

The main problems, then, connected with this fungus are:

- (1) The formation and subsequent behaviour of the gametangia.
- (2) The sexuality of the fungus.
- (3) The germination of the zygosporoes.

METHODS.

All the experiments were carried out on material grown on artificial media. Three per cent. agar, potato-dextrose agar (31), potato agar (31), a decoction of cow-dung with agar, Claussen's medium (32), and Barnes's medium

(32), with and without glucose, were used. On all, zygospores and sporangia were produced.

The cultures were usually kept in diffuse light at temperatures varying from 10° to 24° C., and some were left in darkness.

Living material was examined in Petri dishes, and, mounted in water, on slides. Growing material was kept under observation by placing a slice of the substratum, about a millimetre thick, in a cell on a slide. To keep the atmosphere moist, a few drops of water were placed round the substratum at the bottom of the cell, and the top was covered by a thin glass slip, so that the conditions were similar to those operating in a Petri dish.

For detailed examination, the fungus was fixed in chromacetic acid of medium strength, in Flemming's strong solution diluted with an equal volume of water, in Merkel's fixative, and in Allen's modification of Bouin's fluid. For morphological study, the material was, after washing, transferred to a 10 per cent. aqueous solution of glycerine, to which a drop of dilute acetic acid and two or three drops of saturated aqueous erythrosin had been added. It was exposed in a watch-glass or shallow dish, covered by muslin, until the mixture had reached the concentration of pure glycerine. The material was then transferred to pure glycerine and mounted in glycerine jelly.

For the examination of sections the material was dehydrated and embedded in paraffin by the chloroform method. Microtome sections, usually 4 or 5 μ in thickness, were stained in Breinl's fluid, or Heidenhain's haematoxylin alone, or with orange G, light green, or erythrosin in clove oil. Flemming's triple stain and safranin with toluidine blue were also used.

MYCELIUM.

On media containing no carbohydrate the mycelium is flat and almost invisible. On potato agar, and on media containing dextrose or glucose, the hyphae rise a few millimetres above the surface and may present a woolly appearance. In all cases the growth is more profuse in darkness. At first the mycelium is white, but becomes grey with age.

The hyphae are much branched, superficial, and usually aseptate. Septa are produced, however, near the origin of reproductive structures, and occasionally occur in comparatively large numbers: as many as eleven have been observed on the main hypha near the base of a single sporangio-phore.

At temperatures between 10° C. and 24° C. the mycelium has grown on every medium used, with the exception of one consisting of 5 per cent. gelatin and 30° per cent. glycerine. Three dishes were inoculated, each with mycelium, sporangia, and zygospores, and kept in very diffuse light at a mean temperature of 18° C. No growth whatever occurred within seven

days, so that Namyslowski's results were not confirmed. In similar inoculations on other media, visible growth occurred within twenty-four hours.

By incubating mycelium on various media it has been found that growth increases with increase of temperature up to 25–26° C. Above 26° C. it decreases, and ceases above 32° C.

ACCESSORY METHODS OF MULTIPLICATION.

Multiplication may be accomplished by means of chlamydospores and sporangiospores.

Chlamydospores occurred singly, or in chains of two or three, in any part of the hypha, and were found on all cultures. They are barrel-shaped,

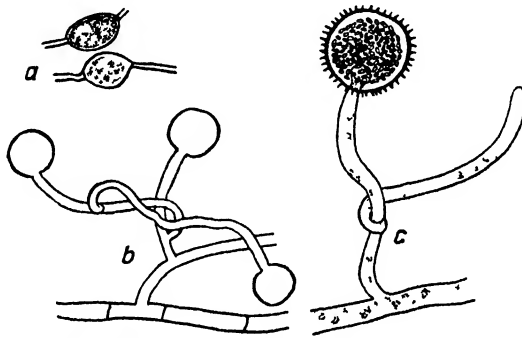


FIG. 1. *a.* Chlamydospores. $\times 367$. *b.* Elaborately branched sporangiophore. $\times 400$.
c. Sporangiophore-branched like zygophore. $\times 533$.

with their long axis in the direction of the hypha, have dense cytoplasm and several nuclei (Fig. 1, *a*). They are liberated by the disintegration of the empty parts of the hypha on either side.

Sporangiospores are formed in very large numbers in sporangia borne by aerial sporangiophores. These are simple, or may be sympodially branched (Fig. 1, *b* and *c*), and may arise on the vegetative mycelium or on the zygophores.

The very young sporangia are colourless and smooth, but they become yellow and spiny before the spores are completely differentiated. Older sporangia, borne on very short sporangiophores, have, when examined in a Petri dish, the appearance of azygospores, and it was not always possible to determine the nature of such structures until they were mounted in water, when they were unmistakable. The spines are apparently crystals of calcium oxalate. They are readily soluble in hydrochloric and insoluble in acetic acid.

When the sporangium is mature, its columella enlarges rapidly, causing the outer wall to burst, and numerous small ellipsoidal spores are liberated (Fig. 2, *a* and *b*). If any moisture be present, these spores rapidly

enlarge, become almost spherical, and put out one or more germ-tubes (Fig. 2, *c* and *d*).

In summer, at a mean temperature of about 21° C., a single spore sown on a potato-dextrose agar (2 per cent. dextrose), and kept in moderately bright sunlight, gave rise to a mycelium producing a few sporangia within 33 hours, and to gametangia during the next 12 hours. Even on

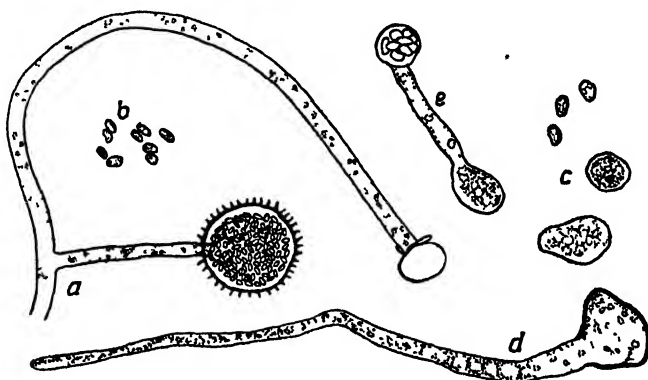


FIG. 2. *a*. Sporangiophore with mature sporangium and distended columella, after dehiscence. *b*. Sporangiospores immediately after liberation. *c*. Sporangiospores enlarging in preparation for germination. *d*. Germinating sporangiospore. *e*. Sporangium at end of a short germ-tube. $\times 533$.

plain agar in winter, at a temperature of 12°–14° C., sporangia were produced on a culture of a single sporangiospore within 54 hours. Fig. 2, *e*, shows a sporangiospore which has produced a sporangium at the end of a short germ-tube. This is very unusual: the mycelium is generally well developed before sporangia are formed.

Sporangiospores were sown on potato-dextrose agar (2 per cent. dextrose), in equal Petri dishes, and incubated in darkness at various temperatures. The following results were obtained:

Temperature.	At end of—	Results.
18°–20° C.	24 hrs.	Germination just begun.
	48 hrs.	Dish nearly half covered by mycelium.
25°–26° C.	24 hrs.	Mycelium well developed.
	48 hrs.	Dish covered by mycelium.
32° C.	24 hrs.	No sign of germination.
	48 hrs.	Small unhealthy-looking germ-tubes were formed.
	96 hrs.	No further growth.

DEVELOPMENT OF THE ZYGOTE.

The zygothores arise as aerial hyphae, each of which produces a lateral branch which curves round and touches the main hypha near the tip. Shortly after contact is made, an outgrowth appears on the main hypha (Fig. 3, *a*), and, as it grows, pushes back the curved branch while

remaining in contact with it (Fig. 3, *b*). Both this outgrowth and the curved branch are the 'progametes' of earlier investigators, and from each a gametangium is sooner or later cut off (Fig. 3, *c* and *d*). Usually the gametangium on the short branch appears first, and fusion occurs shortly after the formation of the second gametangium (Fig. 3, *e-g*). It is probably owing to the brevity of the separate existence of the two gametangia that

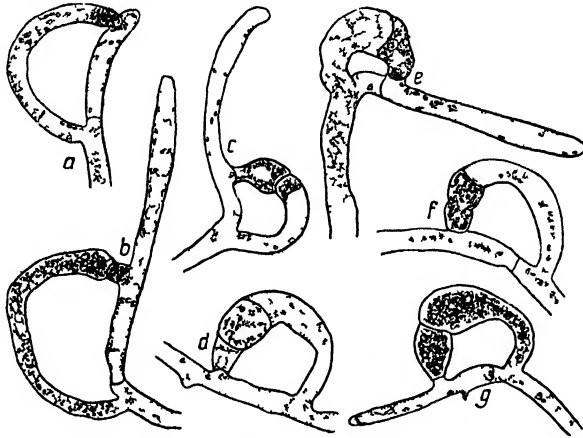


FIG. 3 *a, b*. Outgrowth of short gametangiophore after contact *c, d*. Unequal gametangia. *e*. Zygote immediately after fusion. *f*. Optical section through very young zygote. *g* Zygote with wall beginning to thicken $\times 400$

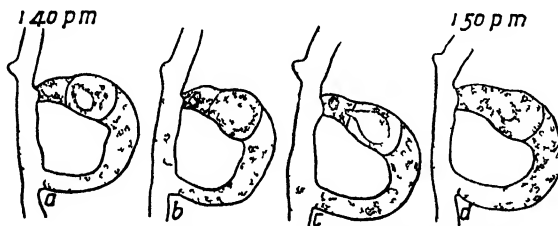


FIG. 4. *a-d*. Stages in the fusion of the gametangia. $\times 400$.

certain observers (Grüber and Grossman) have missed either one or both of them.

Just before fusion, the cytoplasm in the gametangia seems to be differentiated into a denser peripheral and a clearer central area (Fig. 4, *a-d*). The intermediate wall becomes perforated and the two central areas coalesce. The remainder of the separating wall disappears and the zygote is formed. In section, a slight indentation is visible in the very young zygote, showing the place of contact of the two gametangia. The zygote grows rapidly, receiving food mainly from the curved gametangiophore, which usually becomes enlarged at the end, before or shortly after the formation of the gametangium (Fig. 5, *a-e*). By virtue of its position on the

zygophore, and also on account of the formation of a transverse septum just above its place of origin, the curved gametangiophore receives the food supply for the zygote and conveys it through a small aperture in the wall. This part of the wall of the zygote is very distinct later as a smooth, light-brown plate, to which the remains of the gametangiophore are attached,

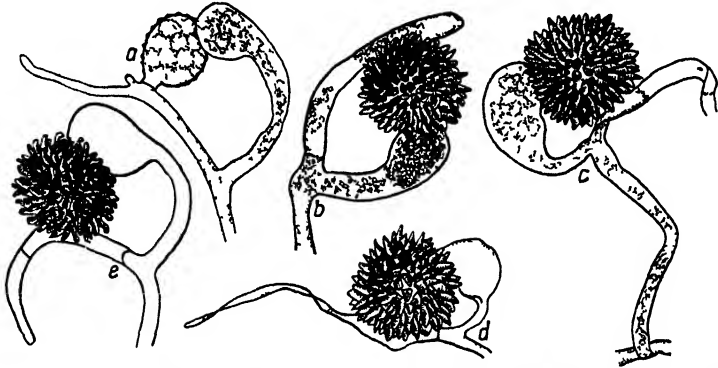


FIG 5 a-e. Progressive stages in the development of the mature zygote. $\times 400$.

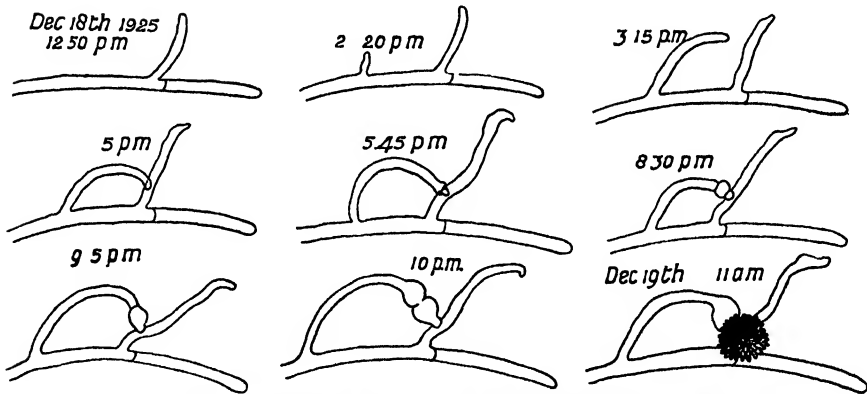


FIG. 6. Stages in the development of a zygote. Slightly diagrammatic.

even after the total exhaustion of the food supply. A similar smaller plate marks the attachment of the other gametangiophore.

The tip of the zygophore becomes destitute of contents long before the zygote reaches maturity. Soon after fertilization the wall of the zygote becomes slightly warty, and within a few hours the warts have grown into spines which are sometimes blunt and sometimes sharp. During the growth of the zygote, and during the disintegration of this echinulate coat, on germination, the spines are often separated into small groups, but they are regularly distributed in the resting zygospore.

Fig. 6 shows the stages and time of development of a zygospore on Barnes's medium (mineral salts with 0.2 per cent. glucose). While this

is the usual mode of development of the zygote, variations are not infrequent.

Sometimes the gametangia are produced on different zygophores (Fig. 7, *a*, *b*), and one branch may produce two gametangia which may fuse with others on independent zygophores (Fig. 7, *c*).

Frequently the zygophore branches sympodially (Fig. 7, *d*), like the sporangiophore, and sometimes it bears sporangia as well.

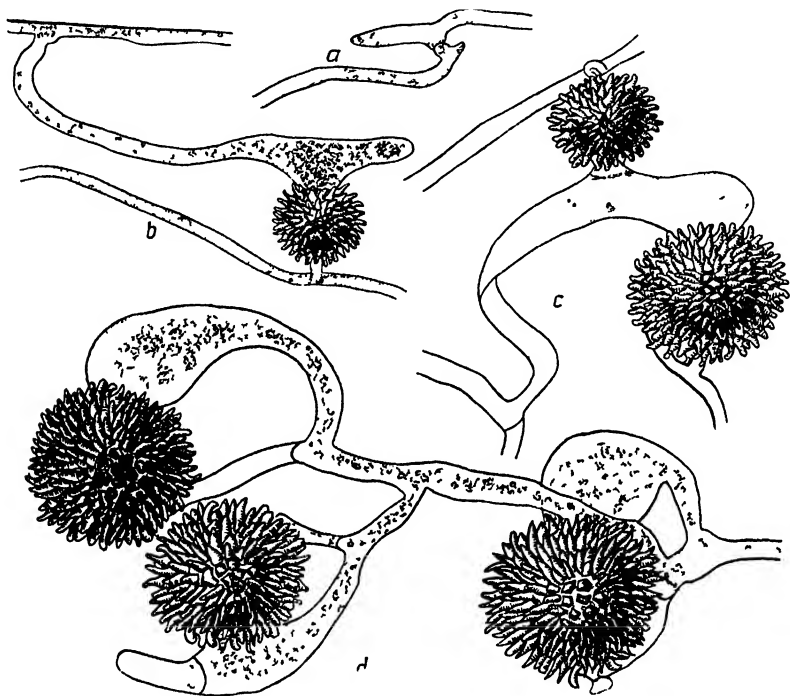


FIG. 7. *a*. Progametangia from different hyphae in contact. *b*, *c*. Zygotes formed from gametangia on different hyphae. *d*. Branched zygophore. $\times 400$.

The relative size of the two gametangia varies. Usually the one on the curved branch is the larger. Occasionally the reverse is the case, while sometimes they appear to be equal. On the other hand the one gametangiophore, for the reasons given above, seems to be constantly larger than the other.

The young zygospore is yellow, but its colour rapidly deepens to brownish black. Within the echinulate coat a very thick wall is developed later.

Practically all the zygospores watched in process of development grew during the night. Zygospore production does occur during the day, but apparently to a lesser extent. In cultures, zygospore zonation, noted by

other investigators, has been observed, and is doubtless due to the difference in the activity of growth during day and night, as they have suggested.

Azygospores have not been seen on any of the media used in the course of this investigation.

GERMINATION OF THE ZYGOSPORE.

At first several attempts were made to germinate zygospores in hanging drops of water and solutions of food substances. In several cases the zygospore burst its echinulate coat, but no germ-tube appeared. Zygo-

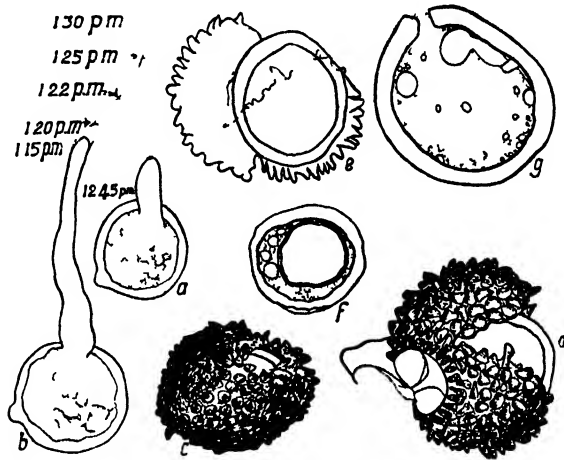


FIG 8 *a, b* Germinating zygospore with growth at noted times. *c, d* Zygospores showing ruptured echinulate coats in preparation for germination. *e*. Optical section through zygospore preparing to germinate *f*. Optical section through zygospore showing thick inner coat and large oil globule. *a-f* $\times 400$. *g*. Disintegrating zygospore $\times 800$

spores from a mycelial culture made on May 1, 1925, were transferred on July 23 to a thin layer of potato-dextrose agar in a Petri dish left in diffuse light. Two days later two spores had germinated. Fig. 8, *a, b*, shows the growth of one of them. Since that time spores one month old have been sown on 3 per cent. agar and have germinated within two days, but results indicate that zygospores from a good food medium, e.g. one containing glucose or dextrose, germinate more readily than those from a mineral salt medium.

Cultures from single zygospores are very difficult to obtain, for sporangiospores often lodge between the spines and no suitable method has been found which will get rid of them without injury to the zygote. One such culture on Claussen's medium produced other zygospores in eleven days. Many single sporangiospore cultures have been made, and none of them have failed to produce zygospores within five days: so it seems reasonable to assume that the culture in question was free from sporangiospores.

All the zygospore germinations occurred in diffuse light, at tempera-

tures varying from 10° to 23° C. On dry media, the zygospores remained in the resting condition. When the medium held moisture and the air was saturated with water vapour, the echinulate coat disintegrated *in situ* (Fig. 8, *c-e*), but when free water was present the zygospore was shot out

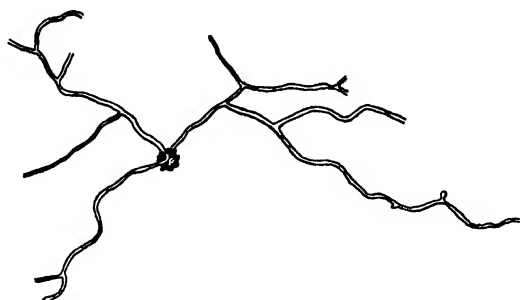


FIG. 9. Mycelium formed from a single zygospore. $\times 60$

of this coat. The large oil globule which constitutes the food reserve of the mature spore could then be seen through the thick transparent coat (Fig. 8, *f*).

<i>Date of Cultures.</i>	<i>Culture Medium.</i>	<i>Date of Transfer of Zygospores to 3 % Agar.</i>	<i>Time of Germination.</i>
Dec. 12, 1925	Potato dextrose-agar (2 % dextrose)	Jan. 11, 1926	24 hours.
	Barnes's medium with 0.2 % glucose	Jan. 11, 1926	24 hours.
	Barnes's medium without glucose	Jan. 11, 1926	About 45 hours.
	Potato dextrose-agar (2 % dextrose)	Jan. 20, 1926	Echinulate coat burst within 19 hrs.; several germ-tubes developed within 38 hrs. (Fig. 9).

When zygospores were subjected to a temperature of 27° C., or over, in bright light, the echinulate coat was ruptured, but germination failed to occur. Sometimes oil-drops were exuded. The rupture of the wall was apparently caused by the expansion of the oil, and was the first sign of disintegration (Fig. 8, *g*).

No zygospore germinations have been observed on media incubated at 24° C. or above that temperature.

RELATIVE NUMBERS OF SPORANGIA AND ZYGOTES.

The ratio of the number of sporangia to that of zygotes varies considerably with the nature of the medium. A series of cultures on different media was left in diffuse daylight for five days at a mean temperature of 15° C., and the numbers of sporangia and zygospores in fourteen equal areas in each dish were then counted and totalled. In the cases marked

with an asterisk the numbers were so few that it was possible to count them accurately; in the others the numbers of zygospores were correct to the nearest hundred.

<i>Medium.</i>	<i>Zygospores.</i>	<i>Sporangia.</i>	<i>Z : S.</i>
*1. Mineral salts (Barnes)	94	6	16 : 1
2. Mineral salts, 0.2 % glucose (Barnes)	2,000	3	670 : 1
3. Potato-dextrose agar (2 % dextrose)	1,600	69	23 : 1
4. Potato-dextrose agar (10 % dextrose)	3,000	110	27 : 1

At the same time similar series of cultures were made in larger Petri dishes, the other conditions being unchanged.

<i>Medium.</i>	<i>Zygospores.</i>	<i>Sporangia.</i>	<i>Z : S.</i>
*1	50	3	17 : 1
2	2,100	10	210 : 1
3	1,600	116	14 : 1
4	3,700	57	65 : 1
4 (in darkness)	2,600	59	44 : 1

On all media, except the first, the difference in ratio was marked. This may indicate that:

- (1) The amount of air in the dish affected the ratio.
- (2) In the larger dishes the peripheral area of the mycelium would be younger than that in the smaller ones, and in this region the ratio might be different.
- (3) There might be no definite relation between the numbers.

The third explanation appeared to be the most probable, for it seemed unlikely that the amount of air, or the age of the mycelium, would affect cases 2 and 3 in the same way, and 3 and 4 in a directly opposite one.

Two cultures made and kept on 3 per cent. agar, under similar conditions, showed striking differences. At the end of sixty-five hours, in one the ratio of sporangia to zygotes was 39 : 4, in the other 1 : 32. The ratio therefore is not governed solely by external conditions. Series of experiments with other media showed correspondingly varied results.

The inference, then, seems to be that the ratio *Z : S* varies according to the medium, but that it is not constant even under given conditions.

Thus Namyslowski's conclusion with regard to the specific constancy of this ratio was not confirmed.

CYTOLOGY.

On account of the very small size of the nuclei in *Zygorhynchus Moelleri*, a satisfactory cytological study could not be completed. Fig. 10, a, shows the nuclei in part of a hypha. In the young zygote there are many small nuclei in a reticulate cytoplasm. Some of these are associated in pairs, but whether this association is accidental, is due to nuclei in division, or depends on the approach of sexual nuclei, cannot be determined. In the curved gametangiophores are small nuclei, which apparently pass in with

the food supply after the fusion of the gametangia. Within the zygote there is apparently nothing to distinguish these nuclei from gametangial ones. The ultimate behaviour of the nuclei has not been observed.

The wall between the zygote and the short gametangiophore becomes thickened very shortly after the gametangia fuse (Fig. 10, *b*). At the earliest stage of development of the zygote a thin wall separates it from the curved gametangiophore (Fig. 10, *b*). Subsequently there is at times open

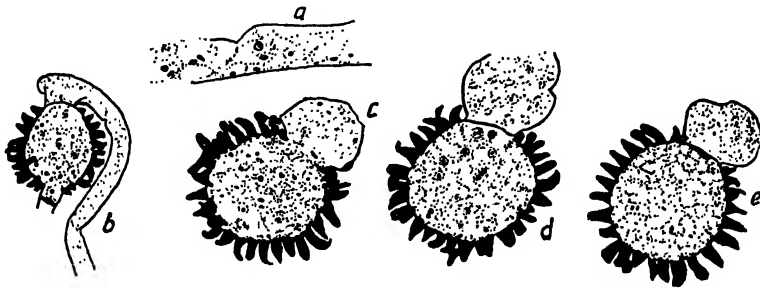


FIG. 10. *a*. Nuclei in hypha. *b*. Section of young zygospore showing the two gametangiophores. *c*. Open continuity between zygospore and enlarged end of curved gametangiophore. *d*. Wall separating zygospore from curved gametangiophore. *e*. Same showing thickening in centre. $\times 550$.

connexion between the two (Fig. 10, *c*), and at others, in the centre of the wall, there is a deposition which suggests a plug (Fig. 10, *d*). From a number of sections examined it would appear that this plug is forced out when the pressure of the food supply is great, and replaced, or re-formed, when the pressure is reduced. The evidence is insufficient to permit any definite conclusion with regard to the exact conditions governing the formation or disappearance of the plug.

SEXUALITY IN *ZYGORHYNCHUS*.

Zygospore formation is undoubtedly the result of a sexual process. Two gametangia fuse and the product of fusion is a resting spore which develops into a new plant when conditions are favourable. The question then arises whether any morphological or physiological difference in sex exists between the fusing elements.

The characteristics usually regarded as criteria of sex in gametes are:

- (1) The larger size of the female cell compared with the size of the male.
- (2) The presence of food reserves in the female and their absence from the male cell.
- (3) The relative physical inactivity of the female and the great activity of the male.

Previous investigators have applied these criteria to the gametangia,

which many of them described as gametes, and even to the gametangio-phores.

It is a matter for consideration whether criteria which are of use in determining the sexual nature of the contents of antheridia and oogonia may, with equal success, be applied to the structures themselves, for in a form such as *Zygorhynchus*, where the gametes are not delimited, any constant distinction between the gametangia might have sexual significance.

Applying the above-mentioned criteria of sex in gametes to antheridia and oogonia, we find :

- (1) There is no essential relation between the sizes of these organs, though the oogonium is usually the larger of the two.
- (2) The oogonium generally receives more food than the antheridium. This is not determined by the relative position of the reproductive structures on the hypha, for the antheridium frequently arises below the oogonium, even when the latter is undoubtedly richer in food material.

Frequently food continues to pass to the oogonium after fertilization, but again the conduction of the food does not depend essentially on the position of this organ. For example, in *Endomyces Magnusii* the oogonium is lateral and the antheridium terminal, while in *Peronospora parasitica* the reverse is the case.

The presence or absence of food reserves does not necessarily affect the relative size of the organs.

- (3) The relative activity of the sexual apparatus varies considerably. In many fungi the trichogyne grows towards the antheridium, as in *Zodiomyces*; in others, such as *Pythium*, a copulating tube is sent out by the male organ.

Thus the one constant difference between the oogonium and antheridium is that food is stored in the former and sometimes supplied to it after fertilization, whereas the latter has no special food reserves and receives no further food supply after the gametes are mature.

With regard to food reserves, the gametangia of *Zygorhynchus* show no difference except such as may be indicated by inequality of size. After fertilization, much more food is conveyed to the zygote through the curved branch than through the short one, but this, except in the case of branches arising on independent zygophores, may well be due to the more favourable position of the former, on the proximal side of the transverse wall. The conduction of food cannot therefore be regarded as evidence that the gametangium cut off from the curved branch corresponds to an oogonium.

Blakeslee recorded the formation of azygospores in *Z. Moelleri* on the short gametangio-phores, never on the curved ones. He considered this to

be an indication of a female tendency, as azygospores are generally produced by unfertilized female cells. Here again a possible function of a gamete is compared with that of a gametangium. Moreover, it is by no means certain that the azygospores were not formed on branches which might otherwise have functioned as normal curved gametangiophores. Further, Vuillemin recorded azygospores on the curved branches of *Z. heterogamus*. In heterothallic Mucors azygospore formation has not been observed, so that there is no evidence in the Mucoraceae to show whether plus and minus gametangia are able to reproduce if fusion fails to occur. Consequently the production of azygospores in *Zygorhynchus* gives no information whatever with regard to the sexual differentiation of the gametangia.

There is no doubt that the gametangia are formed as Vuillemin, Blakeslee, and Moreau have recorded, and that Grüber was able to formulate his very definite statement regarding the sexual differentiation of the gametangia only as a result of his incomplete and misinterpreted observations. He failed to observe the formation of the two separate gametangia, and interpreted the passage of the food supply through the perforation of the wall between the larger gametangiophore and the zygote as a fertilization process.

If, instead of morphological criteria, physiological ones be applied, as in hybridization and parasitization experiments, it is, from results so far obtained, impossible to distinguish the gametangia definitely as plus or minus. Sometimes a gametangium indicates a plus, while another on a similar gametangiophore seems to indicate a minus tendency, but, as Blakeslee pointed out, his experiments were not complete.

On the other hand, since no azygospores have been seen in any of the very large number of cultures made in the course of this investigation, it is impossible, in the case of *Z. Moelleri*, to agree with Vuillemin that heterogamy indicates a tendency towards the total disappearance of sexual reproduction.

SYSTEMATIC POSITION OF ZYGORHYNCHUS.

Because of the inconstancy of the nature of the gametangia, which has already been sufficiently emphasized, *Zygorhynchus* seems to occupy an intermediate position between isogamous homothallic Mucors and such constantly differentiated heterogamous homothallic forms as *Dicranophora* and *Syncephalastrum*.

SUMMARY.

1. *Z. Moelleri* grows readily on artificial media, those containing carbohydrate being more favourable than those containing only mineral salts.

2. With the exception of one medium, on which no growth occurred, zygosporcs were formed in very large numbers in all cultures. Sporangia were also present, but usually in much smaller numbers. There is no constant ratio of the number of zygosporcs to that of sporangia, even when all cultural conditions are the same.

3. Gametangia may fuse with others on the same or on independent zygophores.

4. The morphological differentiation between the gametangia is not constant, and from results so far obtained there is insufficient evidence to show whether any physiological differentiation of sex exists.

5. Mature zygosporcs germinate in diffuse light, at temperatures between 10° and 23° C., on 3 per cent. agar, potato-dextrose agar, and agar with mineral salts. No germinations have been obtained in hanging drops.

6. On account of the small size and large number of the gametic nuclei, their behaviour in the zygote has not been determined.

In conclusion, I wish to thank Professor Dame Helen Gwynne Vaughan and Mr. J. Ramsbottom for their advice and criticism throughout this investigation, which has been carried out at Birkbeck College, and for references to the literature relating to the subject.

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The Mechanical Action of Crustaceous Lichens on Substrata of Shale, Schist, Gneiss, Limestone, and Obsidian.

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With Plates XXII and XXIII and twenty-two Figures in the Text.

IN a recent paper dealing with the mechanical action of crustaceous epiphloeodal lichens on their substratum (1) it was shown that the disintegration of the superficial layers of cork was caused by the expansion and contraction of the lichen thalli firmly and closely attached to that substratum. In the following account an attempt is made to show that some of the disintegration of the superficial layers of rock substrata is due to a similar mechanical action of epilithic crustaceous lichens. In this paper, as in the previous one, details of the structure of the crustaceous species under discussion are omitted, as is also the chemical action of these lichens on the substrata.

I. GENERAL REMARKS ON THE MORPHOLOGY AND DEVELOPMENT OF CRUSTACEOUS LICHENS.

Saxicolous crustaceous lichens spread over the rock surface by means of a peripheral or marginal meristematic zone composed almost entirely of hyphae. In these species there is no lower cortical tissue, the medullary zone being attached directly to the substratum without a rhizoidal or hapteral system. This close attachment to the rock is an important factor in the mechanical action of crustaceous species. The growing fringe or hypothallus may be composed of very dark hyphae. These are practically colourless at the extreme tips, where the walls are more or less gelatinous, and in addition they may be embedded in a very thin mucilaginous film. In some species the hypothallus is white and thick, e.g. *Lecanora parella*. Here the radiating hyphae are massed together, adhering to each other by their gelatinous walls. Normally this white fringe is attached closely to the substratum, but when the lichen is grown under very damp conditions

the hyphae do not group themselves in masses, but grow as separate filaments away from the substratum. When removed from their rock substratum, both dark and light hypothalli show mineral particles attached to, or embedded among, the hyphae. Under very dry conditions, owing to evaporation and consequent contraction of the cortical tissues immediately behind the marginal fringe, a pulling strain is set up in the latter. In the case of the thin, dark-coloured hypothallus of *Rhizocarpon geographicum*, the pull frequently causes a tearing across of the hyphal strands at right angles to the strain, leaving the extreme tips still attached to their substratum. In the thick white hypothallus type this does not occur. The hyphal tissue is torn up from the rock, and, owing to the contraction of the cortex, bends slightly away from the substratum. During this process some small fragments of the substratum may be detached with the peripheral hyphae. Thus the lichen at its margin brings about disintegration on a very small scale. This action can be seen best when the hypothallus is growing over another crustaceous thallus. The individual hyphae or hyphal strands of the encroaching form become firmly attached to the surface of the apothecia and areolae of the lower lichen, and, by the contraction of the tissues of the former, parts of the latter become torn from their substratum and pulled towards the invading species. Each separated fragment then becomes surrounded by the hyphae of the encroaching lichen, and is ultimately incorporated into the thallus of the latter. Such is the fate of the small mineral particles which are separated from the rock below the hypothallus.

Behind this peripheral fringe, green algal cells are found in the thallus, first appearing as small groups encircled by hyphae. These form small irregularities on the surface. They are well seen in *Rhizocarpon geographicum*, the small protuberances being green and the hypothallus black. Owing to the multiplication of the gonidia and more vigorous growth of the hyphae, increase in size takes place until the protruberances become pressed against each other, giving the areolate appearance of crustaceous lichens. Areolation may also be caused by the rupturing of the contracting tissues, or it may be due to the unequal growth of different parts of the thallus (2).

To explain the action of the fully grown thallus is more difficult than to account for the disintegration action at the growing margin. It is known (1 and 3) that when areolae, and more particularly apothecia, are removed from the rock or bark on which they are growing, one finds attached to their lower surfaces thin flakes of their respective substrata. To study the phenomenon in the former case it was not possible, as it was with corticolous lichens, to section the plants *in situ*, except in the limestone species where one decalcified the material with dilute hydrochloric acid. Yet it was desirable for the present investigation to obtain intact the particular

part of the lichen to be studied. This was very difficult except in a few examples, e. g. lichens growing on obsidian. An additional difficulty was that in a number of cases some amount of the substratum, not directly in contact with the tissues, had to be removed with the lichen thallus.

II. PREPARATION OF MATERIAL FOR SECTIONING.

Since the lichens freed of their substrata were too friable to be sectioned by hand, it was found necessary to embed them before cutting, and as serial sections were required the paraffin method was adopted.

The lichen, attached to its substratum, was soaked thoroughly in water, when the tissues became very soft. The part to be removed was then separated off from the surrounding thallus by cutting down to the substratum with a sharp scalpel. When the surface of the rock was smooth and polished the removal of the lichen was simple. Below the edge of the cut part a scalpel was inserted, and by repeated forward and upward movements of the latter the required portion was raised from the substratum. In a number of cases the tissues were practically uninjured. Crustaceous lichens on gneiss and schist were more difficult to free uninjured by this method, since the medullary hyphae penetrated all the little irregularities in the surfaces of these hard rocks. In order to obtain intact the required parts of crustaceous lichens from the softer shales, one had to remove the superficial laminae of the substratum. Often it was found advisable to cut or scrape away these laminae when the material was embedded in the paraffin, and then to re-embed the lichen.

The presence of rock fragments both in and below the crustaceous lichens made it practically impossible to obtain good sections. The amount of tearing and breaking in the sections was an indication of the number of mineral or rock fragments included in the lichen tissues. In addition, there was the difficulty of cutting the carbonaceous material which forms so large a part of lecideine apothecia.

The decalcified lichens from limestone and the freed species from schist, shale, gneiss, and obsidian were fixed with acetic alcohol (three parts absolute alcohol and one part glacial acetic acid), infiltrated and embedded in the usual way.

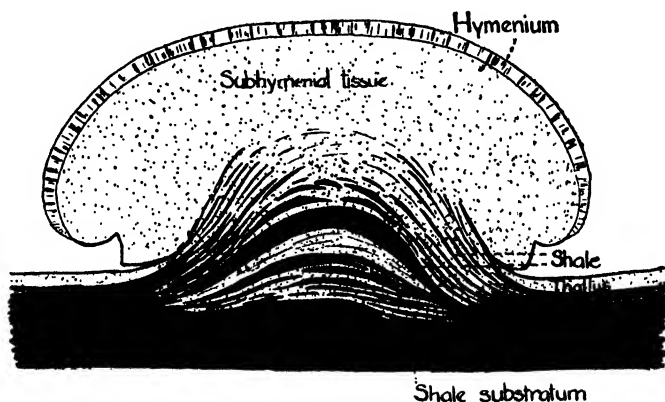
Microtome sections were cut of 12μ thickness, unless thinner ones were required for details of structure. During cutting, staining, and dehydration, some of the rock fragments became dislodged from the tissues, but sufficient remained *in situ* to indicate the general position of the disintegrated pieces of the substratum.

Throughout the investigation Heidenhain's iron-alum-haematoxylin was employed, with Congo red as the counterstain.

III. (a) THE ACTION OF CRUSTACEOUS LICHENS ON SHALE.

(Ordovician Shales, near Barmouth and Aberystwyth.)

In the present account examples of crustaceous lichen action on shale, schist, gneiss, limestone, and obsidian are described. Owing to the lamination of the shale, crustaceous lichens produce on that substratum a somewhat similar effect to that previously described for epiphloeodal crustaceous species on the laminated cork. Because of this it seems advisable to begin



TEXT-FIG. 1 (semi-diagrammatic). Vertical section through an apothecium of *Lecidea* species, showing arching of the shale laminae below. Dotted mass = dense brown hyphal tissue.

with an account of the mechanical action on the shale, particularly on the softer, more laminated type, found at Arthog, near Barmouth.

It has been stated in a previous paper (1) that when an attempt to remove crustaceous lichens from a shale substratum was made by drying gelatin upon the surface of the thallus, the latter came away from the rock bringing with it flakes of shale. These flakes were constantly found below the apothecia, but less commonly below the areolae. This shows that below the apothecia the action was greater and more uniform.

When the large apothecia of such a form as *Lecidea confluens* are removed from the substratum by gently levering up the reproductive bodies, the under-sides of the latter are seen to be arched, and these arches to be lined with plates of shale. A thick hand-section of such an apothecium shows the tissue below the body to be intercalated with shale laminae (Text-fig. 1). These laminae follow the general curve indicated by the lower surface of the detached apothecium. The appearance recalls very strongly the phenomenon observed below the fruiting bodies of epiphloeodal crustaceous species, and this similarity is brought out still more clearly when

horizontal sections of such an apothecium are cut, the thin shale plates forming concentric circles of a light grey colour, between which are packed the dark-brown lichen hyphae. The shale plates so situated do not appear to differ chemically from the rest of the substratum. Frequently one finds that the cavity below the apothecium contains a number of hyphae, the lower extremities of which are attached to the flat substratum (cf. Pl. XXII, Figs. 4 *a* and 4 *b*).

Particularly instructive were some experiments in which *Lecanora atra* and *Lecidea confluens* on shale were treated for some days with concentrated sulphuric acid, after which time the shale was gently washed with water and allowed to dry. In the positions of the former apothecia there were small mounds of the shale. In some few cases depressions were present instead of mounds. When the latter were cut across with a sharp scalpel, below each arched surface there was seen to be a cavity. The hyphae which previously were within the cavity had been destroyed by the action of the acid. There occurred a little fracturing round the base, where the laminae were bent up from the horizontal substratum, but apart from this the curved shale plates were not obviously broken.

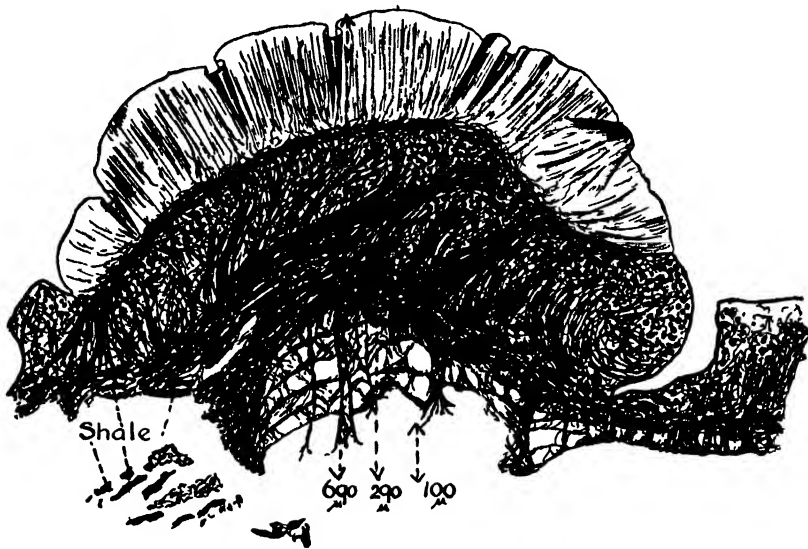
These preliminary experiments seem to indicate that the action of apothecia on a substratum of soft shale is similar to that of the fruiting bodies of lichens on a substratum of periderm, but microtome sections of saxicolous forms show that the action in the former case is probably more prolonged.

Lecidea confluens (Pl. XXII, Fig. 1) and *Lecidea plana* (Pl. XXII, Fig. 2) show the arching of many successive layers of shale, the uppermost layer in both examples being in contact with the hypothecial tissue. In *Lecidea confluens* particularly, the breaking of the section, due to the presence of shale fragments, is well seen. In *Lecanora atra* (Pl. XXII, Fig. 3 *a*) the raised broken shale is also shown, but below the main mass of fragments there is a cavity—indicated by the lower dotted line—in which only a very few hyphae are present (*a*). These hyphae originate in the tissue between the shale fragments above, and enter the cavity by the cracks in the roof in exactly the same way as the hyphae penetrate through the broken periderm arches below the fruiting bodies of epiphloeodal lichens (1).

In *Lecanora sulphurea* (Pl. XXII, Figs. 4 *a*, 4 *b*, 4 *c*, and 4 *d*) one meets with an instructive example of the action of an apothecium on its substratum. In Fig. 4 *a* the three oldest arches (1, 2, 3) show as zones of hyphae and shale, the shale being broken up into small plates, the long axes of which lie parallel with the curve of the arches (dotted lines). Through the fractures the hyphae from the sub-apothecial tissue penetrate into the cavity below, where they are more or less definitely arranged (Pl. XXII, Fig. 4 *b*). In this section there appear narrow strands of hyphae originating at the top and sides of the cavity and stretching down to the floor, while others run at

right angles to these and follow the curve of the roof of the cavity, only approaching the wall at the extreme base (Pl. XXII, Fig. 4 *b*, 1, 2, 3, 4, 5, 6, 7, and Text-fig. 2). The latter strands are sections of successive mats of hyphae, originally on the floor of the cavity, and which, owing to successive pulls from the apothecium above, in the manner already described (1), have been raised from their original position on the substratum.

The scarcity of hyphae between the horizontal layers shows that between the successive pulls only comparatively short periods of time



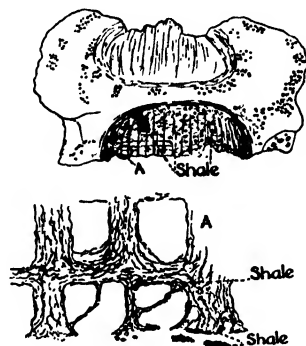
TEXT-FIG. 2 (semi-diagrammatic). Vertical section through an apothecium of *Lecanora sulphurea*, showing the arching of shale fragments and the arrangement of hyphae within the cavity.

elapsed; had they been longer, the tissue in the arch would probably have been more dense. The vertical strands of hyphae connect the substratum with the roof of the cavity, whether that roof be formed of shale fragments (Pl. XXII, Fig. 4 *a*, 1) or of hyphal tissue alone (Pl. XXII, Fig. 4 *b*, 2, 3, 4, 5, 6, 7). It is along these vertical strands that the pulling strain, set up by the expanding gelatinous apothecium above, is transmitted to the floor of the cavity, ultimately causing the latter to become separated from the rest of the substratum. If the floor be composed of hyphal tissue saturated with water, then the hyphal tissue separates from the substratum with little or no particles attached to its lower side. But if the floor tissue be dry at the time when the pull is exerted, and therefore strongly attached to the rock, either shale laminae are pulled up with the hyphae, or the vertical strands, unable to withstand the strain, are torn across. Fresh hyphae grow down from the new roof and become attached to the substratum, whether or no this be a fresh exposure of rock. Where they impinge on that surface the

hyphal strands spread out radially, forming attachment organs similar to the hapteral discs described for *Xanthoria parietina* (4), or the spreading hyphae mat together to form a continuous covering (Pl. XXII, Figs. 4 c and 4 b, respectively, and Text-fig. 2).

On harder shales, such as occur in the Ordovician strata on the coast near Aberystwyth, the effects of this pulling strain, illustrated so well on the soft shales, is not so obvious, for the laminae of the former are not easily torn up by the hyphae. For example, in *Lecanora parella* small fragments only are separated from the substratum with the hyphae, and these become embedded in the dense tissue which ultimately forms in the cavity (Text-fig. 3). In the lower part of the tissue filling the original cavity, indications of the horizontal layers and vertical strands of hyphae can be traced (inset 3), and even small shale fragments are seen adhering to the detached hyphae. But at the apex of the arch, which is often only indicated by the general direction of the hyphae (Text-fig. 3), the horizontal arrangement becomes lost. In this position, too, there is often very little indication of the vertical strands. This example reminds one of the structure of lichens growing on harder rocks than shales; but before passing on to these, one or two examples of the thallus action on shale will be given to show that the action is less powerful than, though similar to, that of the apothecia.

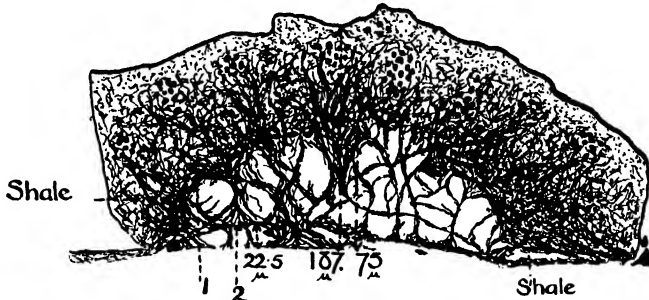
In the section of *Lecanora sulphurea* thallus illustrated in Pl. XXII, Fig. 4 d, and Text-fig. 4 it can be seen that at least two effective pulls have been exerted on the substratum by the thallus. The first of these resulted in the higher and well-marked, though rather broken, arch of shale. This discontinuity is due, firstly, to the friable nature of the shale, and secondly to the expansion of the thallus on absorption of water. The hyphae from the thallus above the arch grow down into the cavity through the gaps in the shale, form strands, and attach themselves to the floor in exactly the same way as described for the apothecium, but the thallus areola, being smaller and less gelatinous than the apothecium, has a much smaller disintegrating action (1). The result of the second pull is not so obvious. This may be due to one of two reasons: either the superficial shale layers had been weathered and were softer than the lower ones, which were therefore less readily detached from the substratum by the hyphae, or the new thinner layers of hyphae forming the covering of the floor of the cavity were less able to function as disintegrating agents than the original thallus,



TEXT-FIG. 3 and inset (semi-diagrammatic). Vertical section of apothecium of *Lecanora parella*, showing the arrangement of shale fragments embedded amongst the hyphae in the cavity. Inset shows arrangement of hyphae at the base of the cavity.

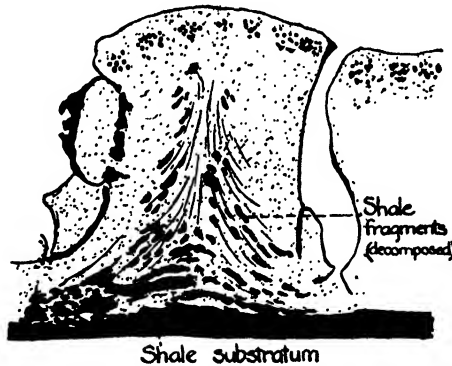
which was more compact and probably more firmly attached to the rock (Text-fig. 4).

Pl. XXII, Fig. 3 *b*, illustrates a vertical section of an areola of a thicker lichen, e. g. *Lecanora atra*, and shows that the arching of the shale is corre-



TEXT-FIG. 4 (semi-diagrammatic). Vertical section through an areola of *Lecanora sulphurea*, showing two successive arches of shale fragments, and cavity with strands of hyphae. 1 = first arch of shale fragments. 2 = second arch of shale fragments.

spondingly greater. The first laminae to be raised have become rather broken (*a* in the figure), and below them occurs a loose network of hyphae (*b*), similar to that found below both the thallus of *Lecanora sulphurea* (Pl. XXII, Fig. 4 *d*) and the apothecia of both these species of *Lecanora*.



TEXT-FIG. 5 (semi-diagrammatic). Vertical section through areola of *Lecanora atra*, showing position of hyphae and shale fragments.

Below this network of hyphae occurs another arch of shale (*c*), which has been broken in the cutting. At *x* in the same figure the hyphae and shale fragments from the base of the areola are drawn up into a point (see also Text-fig. 5). By the present writer this phenomenon is considered important, in connexion with a suggested theory of the origin of the higher fruticose and foliose lichens.

From a study of lichens growing on shales, one concludes that the

mechanical forces operating in these species are the same as those acting in the corticolous lichens, since the former produce in the laminated shales an effect similar to the latter in the superficial periderm layers.

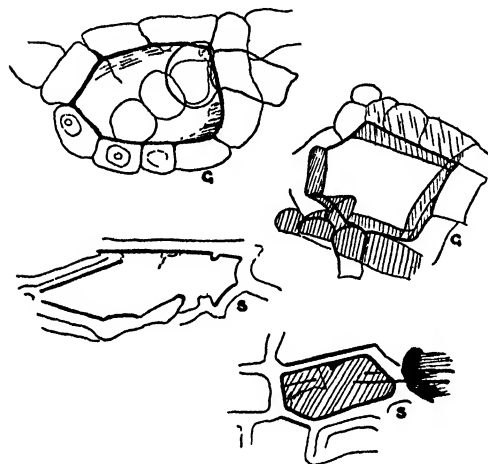
(b) THE ACTION OF CRUSTACEOUS LICHENS ON SCHIST.

(Pre-Cambrian, South Anglesey.)

A few lichens, growing on a schist substratum, were treated with concentrated sulphuric acid in the way already indicated for species growing on shale. Instead of small mounds being exposed on the schist, where the fruiting bodies had been, there were shallow depressions in the rock. Since the surface of the schist is generally uneven, it was not possible to state with certainty whether or not these depressions had been in the rock before the growth of the lichen over the schist, but after examination of sections of the same species as used in the experiments, one was able to conclude that very possibly many of these small hollows were the result of a mechanical action similar to that already described. In cutting the sections of apothecia of these lichens, it was almost impossible to obtain them without tearing some part or other of the sub-apothecial tissues (Pl. XXII, Fig. 5 *a*, *x*). The small mineral particles are distributed throughout much of the tissue, and they are the same as those of which the substratum is composed—namely, quartz and green mica. In vertical sections the region containing the rock fragments has the form of an arch, and commonly there is no definite arrangement of hyphae into strands, comparable with that in *Lecanora sulphurea*. In the illustration of *Lecanora sordida* (Pl. XXII, Fig. 5 *a*), two apothecia are figured growing very close together (*a* and *b*), and consequently a double arch of hyphal tissue, including fragments of the substratum, is shown (indicated by dotted line).

A curious differentiation in staining is obtained in all the sections of lichens grown on schist. Generally speaking, in that part below the apothecium where the majority of mineral particles are embedded, and also in the lower parts of the areolae, the tissue as a whole takes on a purplish tint instead of the usual stain with Congo red. These purple regions rarely extend to the lowest part of the sections. Where the lichen abuts on the substratum there is usually a thin zone of red-stained hyphae, and this tissue contains fewer mineral particles (Pl. XXII, Fig. 5 *a*, *c*, and Fig. 5 *b*, *b*). In the section illustrated in Pl. XXII, Fig. 5 *a*, the purple region is extensive, stretching nearly up to the base of the apothecium, while the red zone *c* is comparatively narrow. The presence of the minerals in the former is indicated by the tearing of the hyphal tissues (*x*), while in the latter there is very little of this. In Pl. XXII, Fig. 5 *b*, an unusual occurrence for lichens growing on a schist substratum is illustrated. The mineral fragments below the two fused apothecia are brown, different chemically from, and softer than,

the rest of the schist. Owing to the softness of the altered schist, apothecia by mechanical action are able to raise the superficial layers as described for the laminated shales. Immediately below the raised altered rock there occurs the purple zone *a*. This is separated from the red region *b* by a distinct, though rather discontinuous, low arch of schist fragments. By comparison with other lichens one is able to conclude that the lower red-stained hyphae are younger than those comprising the purplish regions above, and correspond to the loose or dense networks of hyphae occurring



TEXT-FIG. 6. *Lecanora sordida*. Hyphae in contact with mineral fragments. Heavy lines and shaded portions indicate purple coloration (see text). G = *Lecanora sordida* from gneiss substratum; s = *Lecanora sordida* from schist substratum.

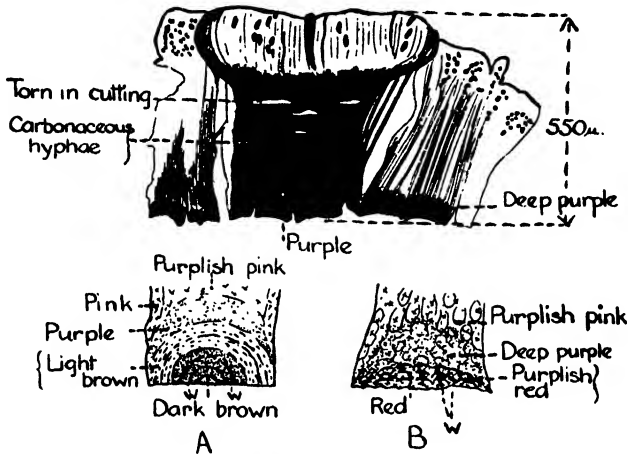
below the older arches formed by apothecial action on the shale. The older purple hyphal tissue has therefore been longer in contact with its included mineral particles than the younger reddish zone. It is the walls of the older hyphae abutting on, or which have at one time abutted on, the included mineral particles that take on the purplish colour. The contents of these cells are not necessarily stained the same colour (Text-fig. 6). Although in contact with the lichen for some long time, yet, as far as one can detect with the microscope, there appears no chemi-

cal alteration in the minerals, even in those parts directly in contact with the purple-stained hyphal walls. Hence one concludes that probably chemical decomposition of the rock minerals by means at the disposal of the lichens is a very slow process, and in schists and shales the mechanical disintegration seems to precede chemical decomposition of the minerals.

The example figured in Pl. XXII, Fig. 5 *b*, is shown merely to illustrate the fact that where the schist is softer, there the lichen is able to raise the superficial layers of substratum. Normally, epilithic lichens on schist are unable to bring about this result, only small isolated fragments being torn up from the rock. These, owing to subsequent expansion and growth of the apothecium (loc. cit.), become scattered throughout the tissue, and rarely show any definite arrangement (Pl. XXII, Fig. 5 *b*). This explains the fact that when lichens are removed from the schist by maceration with concentrated sulphuric acid, small depressions are left in the substratum in the positions of the decomposed apothecia.

In the carbonaceous foot at the base of lecideine apothecia, e.g.

Rhizocarpon geographicum, one finds indications of the arching of the tissues, but very few minerals can be traced. Where the hyphal tissue of either thallus or apothecium abuts directly on the schist, it is very often split up vertically into strands. Frequently at the base of each of these dark-coloured strands may be seen a small purplish region, below which occurs a narrow zone of hyphae stained with Congo red. This splitting into strands is an exact replica of what occurs more generally below the apothecia and areolae of lichens growing on obsidian and gneiss. Within



TEXT-FIG. 7 (semi diagrammatic). Vertical section through an apothecium of *Rhizocarpon geographicum* on schist. Note: arch of non-carbonaceous hyphae (purple); tendency for lower tissue to split into strands. Insets A and B (diagrammatic). Vertical section through strands of lower tissue, showing zones of colour and well-marked basal cell-walls (*w*).

the differently stained regions themselves there appears to be a kind of zonation of the hyphae (Text-fig. 7, insets A and B). This appearance suggests that each zone in its turn has been subjected to pulling strains similar to those previously described. In some cases the hyphae which abut directly on the substratum are brown or slightly carbonaceous, and above these occur the purple and red zones mentioned. Where the hyphal filament tips impinge on the substratum they spread out very slightly over the surface, and remain attached to it by their flattened end-walls (Text-fig. 7, inset B). Very few mineral particles can be detected in such tissue.

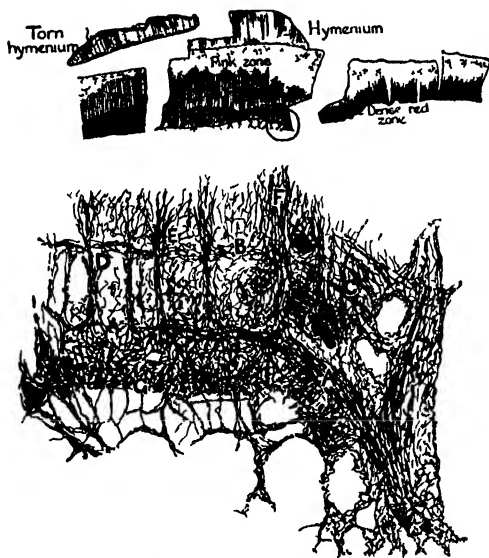
There is a difference therefore between the results obtained by lichen action on shale and schist. In the former the laminae are pulled up into fairly continuous arches below the apothecia. They become broken by continual apothecial and thallial expansion, and through the fractures fresh hyphae penetrate the cavities below. Contact with the minerals of disintegration causes no alteration in the staining properties of the hyphal walls. In contrast, separate minerals, or very small particles of the substratum, are pulled up from the schist, and the subsequent growth of hyphae

from above is uninterrupted by such small fragments, which appear more or less scattered throughout the hyphal tissue. In the hyphal walls which have been in contact for a long time with the included minerals there appears some chemical alteration, but there is no visible indication of decomposition of the minerals themselves. In some species the lower part of the tissues may become separated into vertical strands, each of which appears to be subject to strains similar to those operating in the areola or apothecium as a whole.

(c) THE ACTION OF CRUSTACEOUS LICHENS ON GNEISS.

(Pre-Cambrian Gneiss, North-west Anglesey.)

The gneiss collected from the Pre-Cambrian outcrops at Rhosneigr, north-west Anglesey, is very hard, and consists of large proportions of quartz and a mineral which appears very like apatite, embedded in a very fine, granular, red-brown matrix. Like the shales at Arthog and the schists in south-east Anglesey, the gneiss is very rich in its crustaceous lichen flora.



TEXT-FIG. 8 (semi-diagrammatic). Vertical section through apothecium—broken in cutting—and areolae of *Lecanora sordida* on gneiss, showing hyphae differently stained. Note few hyphae in the cavity below the apothecium. Inset (semi-diagrammatic). Note: (1) Hyphae tend to be grouped in strands—A, B, C with a tendency to run parallel with wall of cavity below; D, E, F, &c., vertical strands. (2) Fragments of disintegrated substratum lie parallel with hyphal arches, e. g. G.

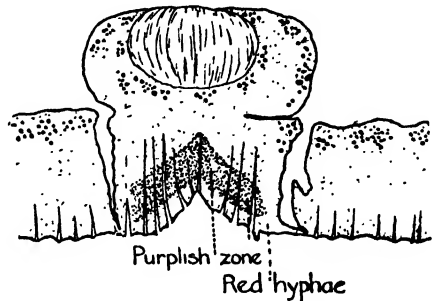
Lecanora sordida is common both on the schist and the gneiss, but on the latter its action is less effective. In stained vertical sections there is the purple-coloured zone described for the species when growing on the schist, but it is less well defined, and also contains fewer mineral fragments. In some material as many as five parallel, narrow, arched, purple bands separated by red-coloured hyphal tissue have been traced. Since, as for the schist-inhabiting speci-

mens, only those hyphal walls in contact with mineral particles are stained purple, it follows that there have been at least five effective periods of disintegration of the gneiss surface in these particular cases. Below the region composed of purple-stained hyphae, or purple and red hyphae in parallel bands, is the more recent growth of tissue which

appears very much the same as that illustrated for *Lecanora atra* (Pl. XXIII, Figs. 7 *b* and 7 *c*). Hyphae grow down from the roof, such as illustrated at *x* in *L. atra*, and attach themselves to the floor of the cavity. These in turn become pulled up, bringing with them minute fragments of the substratum, and form a new roof as at *y* in the same figure. (See also Text-fig. 8.) While the red zone below the apothecia has this loose texture, below the areolae it is a very dense tissue, the individual hyphae of which are wider than in the former case, but the arrangement of the filaments of the latter into vertical and more or less horizontal strands is similar to that so clearly shown in Pl. XXII, Fig. 4 *b*; Pl. XXIII, Fig. 7 *b*. The position and appearance of the minerals in stained preparations is similar to that described for the regions below the apothecia.

As for the crustaceous lichens growing on schist and obsidian, the tissue abutting directly on the substratum may become split up vertically into strands (cf. Pl. XXIII, Fig. 8 *a*).

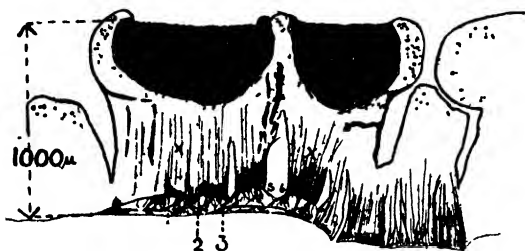
Pl. XXII, Fig. 6, illustrates a vertical section through an apothecium of *Lecanora parella*, and shows particularly well the purple, arched band of hyphae containing the greater number of mineral fragments (*x, x, x*), below which is the dense red mass of hyphae filling the original cavity. Text-fig. 9 illustrates the phenomenon of splitting up into strands of the lower tissues of the areolae and sub-apothecial regions. It also indicates that these strands can be traced through the purplish stained zone as far as the base of the apothecium itself.



TEXT-FIG. 9 (semi-diagrammatic). Vertical section through apothecium of *Lecanora parella* on gneiss. Note arch, purplish zone, and vertical strands of hyphae (see text).

Perhaps the most instructive example of the slow disintegrating action of a lichen on a hard substratum is afforded by *Lecanora atra* when growing on gneiss. All the larger fragments of mineral occur in the cracks of the thallus between the areolae, and from their shape and size it is concluded that they owe their position there to the action of external agencies. Although the thalli of *Lecanora parella* and *Lecanora atra* were the thickest examined during this investigation, and one would expect that their disintegrating effect on the substratum would be the greatest, yet, owing to the hardness of the gneiss, such is by no means the case. Text-fig. 10 and Pl. XXIII, Fig. 7 *a*, illustrate the effect produced by the apothecial action of *Lecanora atra*. The denser tissue (*a* in the second figure), pulled free of the substratum, forms a low cavity and is split up vertically into strands of hyphae. This is shown more clearly and semi-diagrammatically in Text-fig. 10. Each strand is made up of horizontal zones

very similar to those described for *Rhizocarpon geographicum* from the schists, and also very like those of *Aspicilia alpina* on obsidian. As in *Lecanora parella* these strands can be traced to the base of the sub-apothecial tissue. As in all the previous examples, fresh hyphal growth



TEXT-FIG. 10 (semi-diagrammatic). Vertical section through two apothecia of *Lecanora atra* on gneiss, showing vertical splitting (*s*) of basal zones; banded structure of the strands (*b*); general direction of hyphae (*x*); three arches of tissue (1, 2, and 3), and arrangement of hyphae within the arches.



TEXT-FIG. 11 (semi-diagrammatic). Vertical section through areola of *Lecanora atra*, showing vertical and horizontal strands of hyphae building up the lower part of the areola. Note arch of lower medullar hyphal tissue as a whole, and also that of the strands linking up the vertical hyphae.

reattaches the lower lichen tissues to the substratum, and this in its turn becomes separated from the rock by subsequent apothecial action.

Arching of the areola tissues takes place in the usual way. Text-fig. 11 illustrates the arrangement of hyphae in a single areola of *Lecanora atra*. Since very few minerals are disintegrated from the hard gneiss by the thallus action, the successive separations from the substratum are indicated only by the general direction of the hyphae.

The hardness of the gneiss is responsible for the small amount of mechanical disintegration caused by crustaceous lichens growing on its surface.

(d) THE ACTION OF CRUSTACEOUS LICHENS ON OBSIDIAN.

(Yellowstone Park, U.S.A.)

Obsidian is a hard acid rock of the nature of glass, being homogeneous in texture with a vitreous lustre and conchoidal fracture. In some of the obsidian material used for this investigation the rock was well covered with lichens, while other specimens showed new growth on clean and polished surfaces. For the most part the lichens were *Aspicilia* species: e. g.

A. alpina, a doubtful species, possibly *A. calcarea*, and *Rhizocarpon geographicum*. Only those specimens inhabiting the unaltered obsidian surfaces were studied.

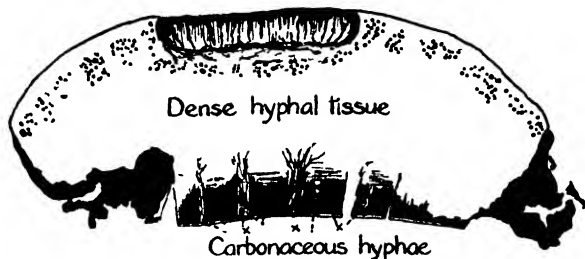
Some of the surfaces unattacked by lichen growth showed fracturing in the superficial layers of rock, and small thin flakes were more or less separated from the main substratum, causing a certain amount of iridescence (4). By the wedge action of the hyphae (1 and 4) these flakes of obsidian may become incorporated in a lichen thallus, but it is not this type of mechanical action that one intends to describe.

When the lichen is completely removed from the polished obsidian surface, the latter is seen to be etched by very minute circular or elongated depressions. This etching was distributed evenly below the older lichens, but was absent on the rock over which the younger thalli were growing. This suggests that it may be the result of chemical action (3). But if this be so, it is rather difficult to explain the fact that the whole of the surface covered is not uniformly changed, instead of the parts between the minute depressions remaining unaltered and highly polished.

When areolae or apothecia of fresh thalli are raised from the substratum, they may either come clear of the obsidian without tearing, or, as is often the case, below the centre the attaching hyphae may become torn near the base, so that the rock originally below the central parts of the areolae or apothecia is left with the torn hyphae adhering. The under-sides of the intact parts of the thallus appear polished, as though obsidian flakes were adhering to the lichen. This very occasionally is the case when the plant has grown over a fractured surface (loc. cit.), but more generally this effect is due to the dense nature of the tissues which form the attachment to the polished obsidian. Even if the very minute flakes from the small depressions were adhering to the under-side of these removed lichen areolae it would be impossible to see them with the magnification of 91 used. It is even difficult to locate such small fragments in thin sections with a much higher magnification. In fact, lichens from an obsidian substratum show very little of this rock embedded in their tissues, but there is usually a large collection of fragments held in the chinks between the areolae and in the peripheral regions of the areolae themselves. As for the lichens on the gneiss, one concludes, from the position and shape of the majority of these particles, that they owe their position to the action of external agencies and not to that of the lichen itself. It is also concluded that even those fragments now within the marginal regions of the areolae were originally in the chinks of the thallus, and have been surrounded by hyphae which have grown out from the neighbouring areolae.

Pl. XXIII, Fig. 8 *f*, illustrates a vertical section through a young apothecium of *Aspicilia alpina*, much of the tissue of which has been torn in the cutting. At *s* the usual arrangement of vertical and horizontal strands is

shown, and at y there appears a repetition of this structure. The explanation of this tissue at y seems to be that at that point the *Aspicilia* thallus had grown over another crustaceous lichen whose intact lower tissues had a similar structure to those of the encroaching species. The unbroken condition of the horizontal strands (x) suggests that the raising of the lichen from the surface of the substratum took place when the former was saturated with water, but the small height and shape are not characteristic of cavities formed under such conditions. In view of experiments made with glass and imitation lichen apothecia, to be described later, and also since it is known that in some cases dry areolae and apothecia can be removed from



TEXT-FIG. 12 (semi-diagrammatic). Vertical section through an apothecium of *Aspicilia alpina*, showing strands of carbonaceous hyphal medullary tissue at base. Note banded structure of the strands, also thin-walled lowest zone abutting on substratum. x = hyphae arising in medulla above strands and stained brilliantly red with Congo red.

the obsidian without tearing of their hyphae, one concludes that in all probability such low arches are the result of sudden separation from the substratum during dry periods. Such separation of dry imitation apothecia from a glass surface can be brought about by sudden alterations of temperature, causing unequal expansion and contraction of the lichen and substratum.

Amongst the hyphae in these lower regions there are only a very few minute flakes of obsidian, showing that there is very little disintegration.

Pl. XXIII, Figs. 8 *a*, 8 *b*, 8 *c*, 8 *d*, 8 *e*, illustrate the splitting of the medullary tissue into strands already mentioned in connexion with schist- and gneiss-inhabiting lichens. The lower part of the strands consists of dark-coloured hyphae, closely packed together (x in Figs. 8 *a*, 8 *b*, and 8 *f* of Pl. XXIII). There is a zonation of these dark hyphae, but it is poorly illustrated in Pl. XXIII, Figs. 8 *a*, LL, and 8 *b*, x . Pl. XXIII, Fig. 8 *c*, illustrates a horizontal section near the base of these vertical strands where they are in close proximity with each other, while Figs. 8 *d* and 8 *e*, taken from regions higher up, show that there they are more widely separated, and in the gaps between them thin-walled hyphae, stained brilliantly with Congo red, are present. These hyphae originate in the medullary tissue above, grow between the strands, and reattach the lichen to the substratum, filling in the gap left between the base of the strands and the rock (Text-fig. 12).

The dark hyphae may put out fresh growths from the base of the strands and reattach the latter in this way. In any case, owing to the small height of the cavity, the amount of growth required to fill it is very small. Since it is generally the more superficial parts of the lichen which expand most on absorption of water, the upper parts of the vertical strands are more widely separated from each other than those nearer the substratum. The carbonaceous hyphal walls are incapable of stretching to any extent, and, when there is exerted a strong pull from the expanding tissue above, this inelastic tissue tends to split into strands.

As has been already stated, only very few and minute obsidian fragments can be traced in the tissue of the compact dark strands, but the fact that they do occur at all at varying depths shows that, in all probability, mechanical force functions to some extent. While not excluding the possibility of chemical attack as being the cause of the etching effect previously mentioned, the suggestion is made that indirectly it might owe its origin to the contraction of the lichen tissues, followed by the sudden separation of the hard carbonaceous material from the substratum to which it is so closely attached. Below the younger parts of the lichens the dense carbonaceous tissue is not present, and there such mechanical action could not be very pronounced. This may explain the absence of etching on the obsidian surface below the younger parts of these crustaceous thalli.

As for the Pre-Cambrian gneiss, disintegration of the obsidian by lichen action is very small owing to the hardness of the rock.

e. THE MECHANICAL ACTION OF CRUSTACEOUS LICHENS ON CARBONIFEROUS LIMESTONE. (The Great Orme.)

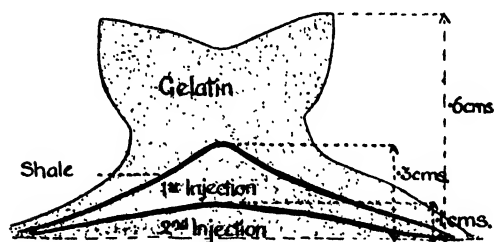
It is known that some chemical reaction between endolithic thalli and limestone does occur, but its exact nature has not been ascertained. It is probable that a certain amount of similar chemical reaction goes on between epilithic species and the same substratum, but one is able to conclude from the structure of the thalli of several of these epilithic species that they also exert a mechanical action on the limestone similar to that exerted by those lichens living on shale, schist, gneiss, and obsidian.

IV. EXPERIMENTS WITH GELATIN AND DIFFERENT ROCK SUBSTRATA.

Experiments with gelatin, similar to those described in connexion with the mechanical action of corticolous lichens (1), were carried out, but in these the artificial apothecia were made to adhere to the different rock surfaces. After the artificial apothecia had been fixed to the substratum, either a short period of one or two hours or a long one of six to twelve hours was given

in order to allow both the adhesive gelatin to set and the surface layers of gelatin to dry a little before immersion in water.

(a) *Soft shales (Ordovician)*. After immersion for about half an hour of the artificial apothecia adhering to a substratum of soft shales, an arching of the rock laminae below some of the 'apothecia' took place. In order to bring about this same result for others, a longer period of immersion was necessary, but this was probably due to the fact that in these cases harder shales had been used. The dome of shale was visible from above through the 'apothecial' gelatin, while it could also be seen in profile from the side of the model of the fruiting body. In the surface view a few cracks in the



TEXT-FIG. 13 Vertical section through imitation apothecium, showing raised shale laminae (see text).

arch of shale were visible, but of these there was no definite arrangement. Round the base of the dome, where the laminae had become separated from the rest of the substratum, a circular crack was usually present. After a long period of immersion in water all these cracks

were observed to widen. This

shows that the area of the

walls of the cavity, to which the laminae of shale were adhering, increased as the gelatin imbibed more water. Where the arching of the gelatin was particularly good, fresh gelatin sol was injected by means of a very fine pipette into the cavity below the raised laminae. The injected gelatin represented the new growth of hyphae in the cavity formed as a result of apothecial expansion. This fresh gelatin was allowed to set and the whole was re-immersed in water. After a second injection, followed by immersion, a section of the whole was cut. This showed two separated arches of shale laminae, in between which, and below the lower, the injected gelatin was present (Text-fig. 13). It will be noticed that the expansion of the gelatin of the second injection had not caused a third shale layer to be raised. This was probably due to the gelatin, of which the 'apothecium' was formed, being thoroughly saturated with water, after which no further expansion could take place. The sections of such artificial apothecia recall very strongly those of lichen fruiting bodies with included laminae (Plate XXII, Figs. 1, 2, 4 a).

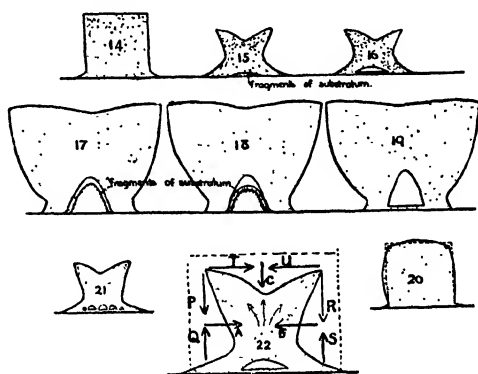
(b) *Gelatin on Pre-Cambrian schist and gneiss and Ordovician hard shales*. Only small isolated fragments of such hard rocks as schist, gneiss, and hard shales were disintegrated from the surface by the action of artificial apothecia when these rocks were used as substrata. These particles remained firmly attached to the walls of the cavity. Unless the whole apothecium had become thoroughly saturated after a long period of immer-

sion in water, the substratum was usually dry below the arches, both in these and the experiments with the softer shales. The disintegration of small fragments of the harder rocks by the mechanical action of the swelling gelatin is exactly similar to the phenomenon observed below living lichens on these rocks. Under natural conditions, owing to the repeated expansion and contraction of the apothecial and thallial tissues, and to the new development of hyphal tissue within the created cavities, these small fragments become scattered (Plate XXII, Fig. 5 a).

In some of the series of experiments, before immersion in water, time was allowed for a certain amount of shrinkage of the artificial apothecia to take place. Below the central regions of nearly every 'apothecium' thus treated, a small cavity appeared to be formed (Text-figs. 15 and 16). After immersion of the material for a short time, in the place of these small cavities appeared the usual arches of gelatin, to the walls of which fragments of substratum were attached (Text-fig. 17).

Other examples showed the original small cavity on top of the larger arch of gelatin (Text-fig. 18), while an occasional one showed only an enlarged cavity with no layer of adhering substratum particles (Text-fig. 19). Sections of these 'apothecia' were cut, and they showed the original small cavity was caused either by the gelatin in the centre becoming free of the substratum with very few rock fragments adhering, or by the gelatin splitting across near the base, leaving a thin film of gelatin adhering to the rock (Text-figs. 15 and 16). Since the gelatin above the split had not been in contact with the substratum, no fragments of the latter were attached to it. The disappearance of the majority of such cavities on immersion of the material in water was due to the film of gelatin, formerly attached to the substratum, being drawn up into the now enlarged original cavity. To the lower side of this film small fragments of the substratum were attached (Text-fig. 17). Where the film was not completely in contact with the roof of the original cavity the latter appeared superimposed on that formed by the raised film of gelatin (Text-fig. 18). Text-fig. 19 illustrates the condition where the gelatin film was not detached at all from the substratum and the original cavity merely expanded as the gelatin imbibed water.

The suggested explanation for the formation of the cavity below the



TEXT-FIGS. 14-22 (diagrammatic). Vertical sections through artificial apothecia (see text). (Fig. 22 shows twice the magnification of the other figures.)

'apothecia' when the latter were dried is as follows: On contraction of the gelatin, strains were set up in the models of apothecia. At first the sides and upper surface bulge slightly before becoming concave (Text-fig. 20). According to Hatschek (6) this is because the exposed ridges suffer first the loss of water, and consequently are the first to contract. These ridges form a stiff framework for the mass of gelatin. On evaporation of water from the sides and upper surface these contract and sag inwards (Text-fig. 22). The unexposed gelatin near the substratum does not lose much water by evaporation, and therefore little contraction takes place, the gelatin in this region remaining soft and pliable. As a result of such different contractions the gelatin suffers a kind of pinching (Text-fig. 22, A, B), which forces the gelatin upwards. Since all the particles in the mass are in connexion with each other, there is a pull exerted on the lower, soft gelatin. At a certain point the strain disappears by the sudden separation of two layers of gelatin near the base, or by the separation of the adhering gelatin from the substratum, with or without adhering fragments of the latter. The cavity, therefore, is a vacuum which increases in size when water is absorbed by the gelatin 'apothecium'. The adhering film—when such is present—is therefore probably drawn up by suction into the cavity.

It has been mentioned that even when the experiments have been immersed in water for some time the substratum below the arched gelatin is generally dry. A similar state of affairs frequently prevails below living apothecia, for during maceration experiments (e.g. *Lecanora parella*), in which prolonged immersion in acids and water was required, one frequently found, even when the upper parts and nearly all the thallus tissues were soaked and half decomposed, that in the arch below the apothecia the hyphal strands were apparently dry and untouched by the acid. It is this feature of the lichen tissues which plays an important part in the mechanical disintegration described for the various lichens, for dry lichen tissues adhere far more tenaciously to their substrata than do similar tissues saturated with moisture. The expansion of the apothecium taking up water brings about the formation of a cavity immediately below it, or it enlarges one already there. As a result, in all probability, a partial vacuum at least is created, and at the same time there is a strain on the hyphal strands attached to the substratum. These operating together probably account for the disintegration of the substratum in these positions.

(c) *Gelatin on glass.* In place of obsidian, glass slides were used as substrata for the artificial apothecia, but in all the experiments which were conducted under the same conditions as those described above, no chipping of the glass or raising of glass fragments occurred below the 'apothecia'. On continued drying, cavities were formed in the gelatin near the base, or between the gelatin and the glass (loc. cit.). In many cases, however, in the place of one large cavity several smaller ones were present (Text-fig. 21).

When the experiments with gelatin and glass were brought indoors out of the heat of the sun, or taken from the air-oven, and suddenly cooled by immersion in cold water or by being placed on a cold table, a sudden separation of the dried gelatin from the glass below the central regions of the apothecia took place (loc. cit.). This was often accompanied by the formation of a circular crack in the gelatin round the base of the 'apothecium'.

As far as one can judge from observations and experiment, disintegration by lichen action of smooth hard surfaces—e. g. obsidian, quartz—does not take place as a result of the ordinary expansion of the apothecial tissues, but more probably by their sudden separation from the substratum, although of this experimental proof has not been obtained.

(d) *Imitation thalli on different substrata.* Gelatin films on different rock substrata were cut so as to represent areolate thalli of crustaceous lichens. These were then subjected to conditions similar to those under which the experiments with the artificial apothecia were conducted, but though the results were similar they were much less marked, probably owing to the thinness of the film compared with the thickness of the 'fruiting bodies' (cf. (1)).

When such cut films were allowed to dry on glass, instructive results were obtained. Many of the films showed that the separation of the gelatin from the glass and chipping of the substratum occurred first below the chinks in the gelatin and spread from these parts to the regions below the areolae, and, owing to the conchoidal fracture of the substratum, split the surface layers deeper below the latter. When all the gelatin was removed, the surface of the glass was ridged below the chinks in the original film of gelatin and slightly hollowed out below the areolae. The absence of this phenomenon in some of the experiments was probably due to incomplete adhesion of the gelatin to the polished glass surface.

An account of the action of contracting gelatin films on a shale surface has already been given (4). The substratum below the periphery of the film becomes torn from its position and raised into the air. Continued drying of the gelatin causes the disintegration of the surface layers to spread inwards from the marginal regions. On moistening the gelatin, the raised parts, with their adhering fragments, sink and become pressed back into their original positions. This is what tends to occur at the growing fringe of crustaceous lichens (loc. cit.) and also at the periphery of each individual areola and apothecium. It is below the periphery and chinks in artificial thalli on a shale substratum that cracks in the laminae first appear; it is also below these same parts that the glass surface is first disintegrated, and it has also been noted that it is below the chinks in epiphloeodal crustaceous lichens that the periderm substratum is often ruptured (1). These illustrations show the effect on the substratum of alternating strains set up by the

adhering lichen above it. It is quite possible that some of the smaller fragments of obsidian and gneiss, occurring in such quantities in the cracks of the thalli growing on their surfaces, owe their position there to lichen action.

The results of these simple experiments indicate that the disintegration of rock surfaces described for crustaceous lichens is the result of mechanical forces similar to those operating in corticolous lichens.

SUMMARY.

1. At the growing margins of crustaceous lichens a small amount of disintegration of the substratum occurs.

2. Sections of crustaceous lichens show that for some substrata disintegration below the apothecia is very pronounced, but below the areolae it is less obvious. This is seen most clearly when shale, schist, or even a crustaceous lichen forms the substratum, but less well when the harder rocks, such as obsidian and gneiss, function in this capacity.

3. The tissues below the apothecium are arched to form a cavity, to the walls of which the disintegrated fragments adhere. Into the cavity hyphae grow from the surrounding tissues, and of these hyphae there is usually a definite arrangement. This arrangement is more clearly seen in the earlier stages before the hyphae become densely packed.

4. The swelling gelatinous apothecial or thallial tissue exerts a pull on the substratum, to which it is firmly attached. When the strain reaches the limit which either the lichen tissue or the substratum can sustain, a break in the one or the other occurs, and arching of the thallus follows as a natural result of the expansion of the tissues above. Thus the physical properties of the substratum—namely, hardness, lamination, &c.—determine the amount of mechanical disintegration which can take place in this way.

5. Arching of the lichen tissues, accompanied by disintegration of the substratum, may be repeated again and again, as often as the expansion of the upper lichen zones, thallial or apothecial, are capable of exercising forces of sufficient strength.

6. From experiments made with artificial apothecia it is concluded that separation from the substratum of hypothecial tissues may also take place as a result of contraction of either the fruiting bodies or the underlying rock. Though disintegration of some substrata below the apothecia may take place as a result of these contractions, it has not yet been proved experimentally for obsidian and glass.

7. As for corticolous species, disintegration of the substratum below the growing margin and the periphery of each areola is considered to be the result of alternating expansion and contraction of the lichen tissues.

8. Experiments with gelatin models of apothecia and thalli on the different rocks as substrata support the theory that the disintegration described is mechanical, and the same as that described for epiphloeodal, crustaceous lichens.

I am indebted to Mr. Kempin, M.A., of the Botanical Department, Oxford, for the photomicrographs which serve as plate illustrations. My thanks are due to Professor W. E. S. Turner, Sheffield University, for supplying me with the obsidian material, and also to Mr. Hebden for his help in the identification of the lichens.

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EXPLANATION OF PLATES XXII AND XXIII.

Illustrating Miss E. J. Fry's paper on the Mechanical Action of Crustaceous Lichens.

PLATE XXII.

Figs. 1-4. *Lichens from Shale.*

Fig. 1. Vertical section of apothecium of *Lecidea confluens*, showing arching of the tissues and shale below.

Fig. 2. Vertical section of apothecium of *Lecidea plana* (Nyl.), showing arching of tissues and shale below.

Fig. 3 a. Vertical section of two fused apothecia of *Lecanora atra*, showing arching of tissues and shale. *a* = hyphae in cavity below.

Fig. 3 b. Vertical section of areola of *Lecanora atra*. *a* and *c* = fragments of shale; *b* = loose network of hyphae; *x*: see text.

Figs. 4 a-c. Vertical section of apothecium of *Lecanora sulphurea* (Ach.), showing arching of shale and tissues, and hyphal development within the cavities (see text). *x*' = hyphal strands.

Fig. 4 d. Vertical section of areola of *Lecanora sulphurea*, showing arching of tissues and network of hyphae in cavity.

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Figs. 5 *a* and 5 *b*. *Lichens from Schist.*

Fig. 5 *a*. Vertical section of two fused apothecia (*a* and *b*) of *Lecanora sordida*. *x* = tears in section; *c* = zone of red-stained hyphae. Dotted line = limit of region containing schist fragments.

Fig. 5 *b*. Vertical section of two fused apothecia of *Lecanora sordida*. *a* = zone of purple-stained hyphae; *b* = zone of red-stained hyphae.

Fig. 6. *Lichen from Gneiss.*

Fig. 6. Vertical section of apothecium of *Lecanora parella*. *x* = limits of purple-stained hyphal zone.

PLATE XXIII.

Fig. 7 *a-d*. *Lichens from Gneiss.*

Fig. 7 *a*. Vertical section through parts of two apothecia of *Lecanora atra*.

Fig. 7 *b*. Vertical section through hyphal network within the cavity below apothecium of *Lecanora atra*.

Fig. 7 *c*. Vertical section through hyphal network within the cavity below apothecium of *Lecanora atra*.

Fig. 7 *d*. Vertical section through four areolae of *Lecanora atra*.

Figs. 8 *a-f*. *Lichens from Obsidian.*

Fig. 8 *a*. Vertical section through apothecium of *Aspicilia alpina* (Somm.). *L* = banded structure of strands; *x* = splitting of medulla into strands.

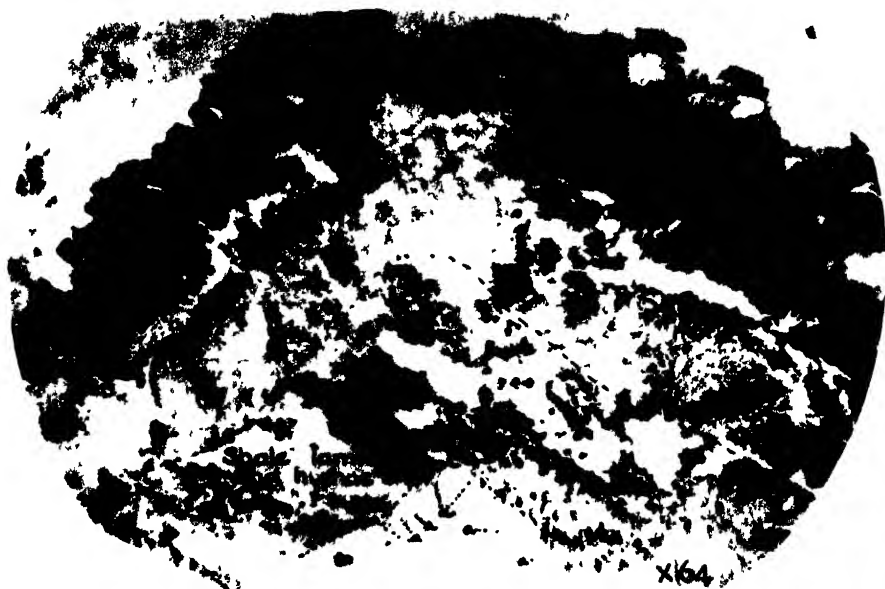
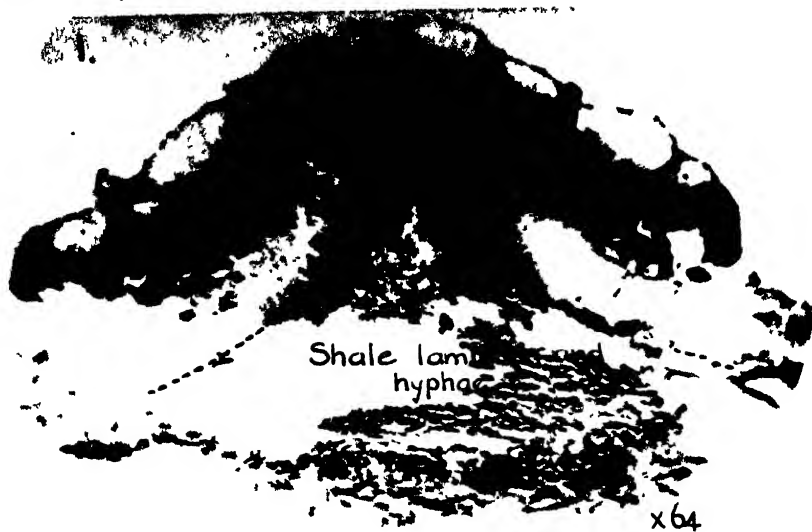
Fig. 8 *b*. Vertical section through areola of *Aspicilia alpina*. *x* = banded structure of vertical strand.

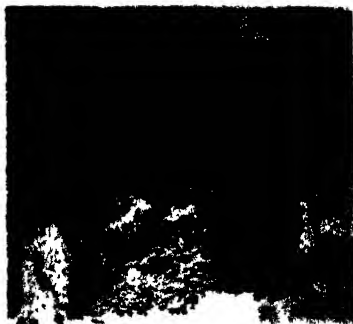
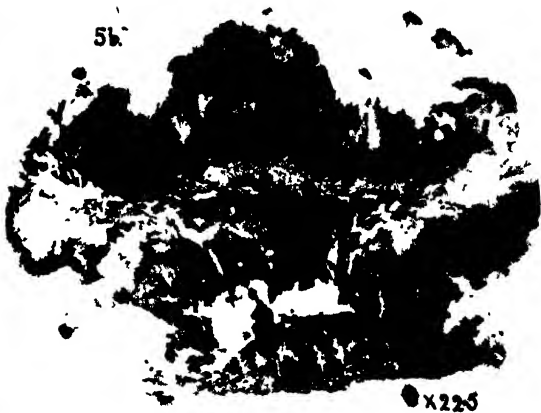
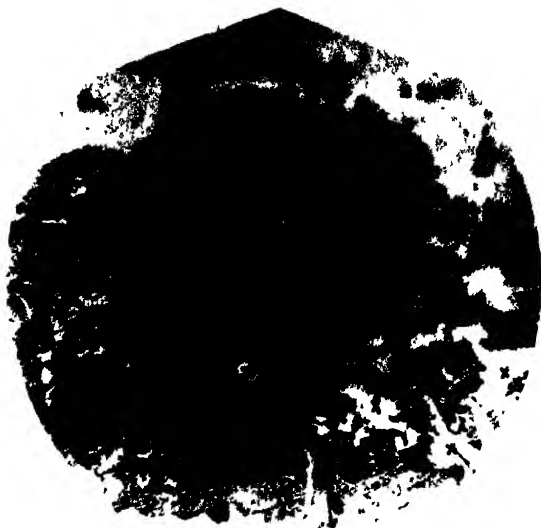
Fig. 8 *c*. Horizontal section at base of apothecial or areola attachment of *Aspicilia alpina*.

Fig. 8 *d*. Horizontal section higher up in same tissue as 8 *c* of *Aspicilia alpina*.

Fig. 8 *e*. Horizontal section still higher up in same tissue as in 8 *c* of *Aspicilia alpina*.

Fig. 8 *f*. Vertical section of apothecium of *Aspicilia alpina*. *x* = medullar tissue splitting; *y* = position of older lichen (see text); *z* = hyphae within original arch.

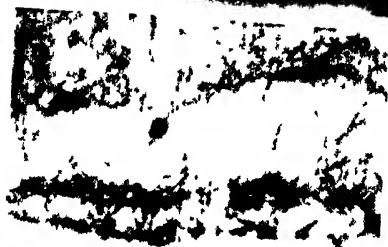




7a



7b x 43



7e x 43



x 43



x 43

8b x 43

8c x 30



On the Occurrence of Two Ovules on Araucarian Cone-scales.

BY

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With Plate XXIV and eight Figures in the Text.

THE interesting family Araucarineae, including the two genera *Araucaria* and *Agathis*, has been the subject of much investigation by various authors. This family derives special interest from the fact that it has been considered by different authors respectively to be the most primitive and the most recent of Conifers (1).

One of the more recent accounts of the family is that by Seward and Ford (2), to which work the reader may refer for a detailed and a general treatment. R. B. Thomson (3) has also treated the subject exhaustively from an anatomical point of view. I shall, therefore, only record here certain as yet unobserved facts for what they may be worth.

The material examined consisted of the female cones of the following species: *Araucaria montana*, *A. Rulei*, *A. Cookii*, *A. imbricata*, *A. excelsa*, and *A. Bidwilli*.

GENERAL EXTERNAL FEATURES.

Araucaria montana. The cone in external appearance closely resembles that of *Araucaria Cookii*. The transition from sterile scales at the base of cone to the normal fertile scales is very gradually effected in a curiously suggestive manner. At the extreme base we have the completely sterile scale with no signs of a 'ligule' or ovule. Then we come to scales with poorly developed, yet two distinctly separate, 'ligules'. Working upwards the two 'ligules' gradually become larger and more prominent. Each assumes a characteristic bulged shape as if each bore an ovule. The two, so to say, ovule-bearing structures gradually approach each other and finally 'fuse' into one, giving the normal one-seeded type of scale characteristic of the genus (Pl. XXIV, Fig. 1).

This transition from one to the other condition is effected in about

a dozen or so scales, the rest of the cone above being of the usual type frequently fertile to the very tip.

This interesting feature at first sight seemed to be abnormal and exceptional for this particular species, but an examination of additional material of other species showed the same character more or less in practically all of them, although in none so well pronounced and definite as in this endemic species from New Caledonia.

At the apex of the cone, evidently owing to close packing, the various scales are only free distally, having grown *en masse* at the base. It is not uncommon to find instances of 'fusion' involving as many as 4-6 scales. The 'two-ovuled' scales from the base of the cone are distinctly not the result of this sort of coalescence. The transitional stages seen in Pl. XXIV, Fig. 1, clearly illustrate this.

Araucaria Rulei. This New Caledonian species also showed the 'two-liguled' feature very clearly. The transition is, however, less gradual, and because of the much smaller size of the scales, as compared with those of *A. montana*, the 'two-liguled' feature is not so prominent a character as in that species. Of the species examined by me, only these two, both New Caledonian, show the transition with striking clearness (Pl. XXIV, Fig. 7).

Araucaria Cookii. Here the transitional stages are not as well marked as in the above two species. The 'ligule' appears first in a rather diffuse form covering a considerable part of the bract surface and with fringed free margins. From this it gradually gets more and more restricted to the median region, ultimately passing into the normal fertile form. The 'ligular' tips, however, present various suggestive shapes.

Araucaria imbricata. In this species, as in others at my disposal, except *A. montana* and *A. Rulei*, two 'ovules' are hardly recognizable externally. Two 'ligules' can often be recognized free distally from the bract surface.

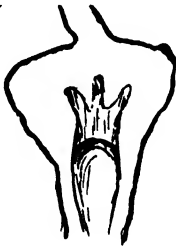
Araucaria excelsa. This species closely resembles *A. Cookii* in the external shape and form of the scales. In transitional scales the 'ligule' is often markedly trifid at the apex (Text-fig. 1, and Pl. XXIV, Fig. 5).

Araucaria Bidwilli. Owing to lack of adequate material of the transitional region, it is impossible to say definitely whether the same condition exists here or not. However, one or two features of the scales are worth noting.

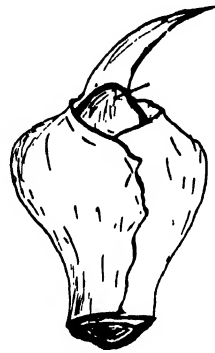
The wings of the huge cone-scales are frequently turned in over the median ovule, forming a sort of an extra protective covering, the ligule being exerted (Text-fig. 2, and Pl. XXIV, Fig. 2). It is interesting to find the same thing in the mature cone-scales of *Cunninghamia sinensis*.

I now pass to the internal anatomy of the cone-scales, specially those of the transitional regions, limiting my observations mainly to the vascular supply.

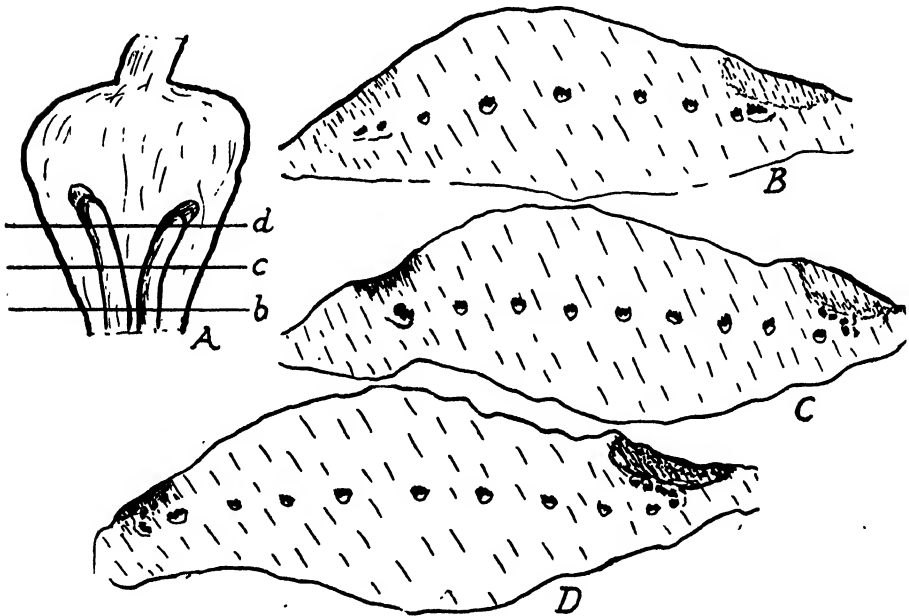
Araucaria montana. The following account is based on microtome sections. Text-fig. 3 shows camera lucida sketches of sections of a scale



TEXT-FIG. 1. *Araucaria excelsa*. A cone-scale from the transitional region showing trifid ligule.



TEXT-FIG. 2. *Araucaria Bidwilli*. A cone scale with both wings folding over the median ovule. The 'ligule' can be seen.



TEXT-FIG. 3. A-D. *Araucaria montana*. A. Cone-scale illustrating the beginning of transition from 'two-ovuled' to the normal, 'one-ovuled', condition. B, C, and D are camera lucida sketches representing sections at levels *b*, *c*, and *d* respectively.

representing the early stages of the transition: the two ligules being feebly developed and fairly far apart.

• It is evident that there are two distinct 'ovular tissues' indicated in the internal anatomy, corresponding in position to those represented externally

by the two 'ligules'. Each of these tissues has its own 'inverted' vascular supply (Text-fig. 3, D). One of the 'ovular' tissues is apparently more developed than the other (Pl. XXIV, Fig. 6).

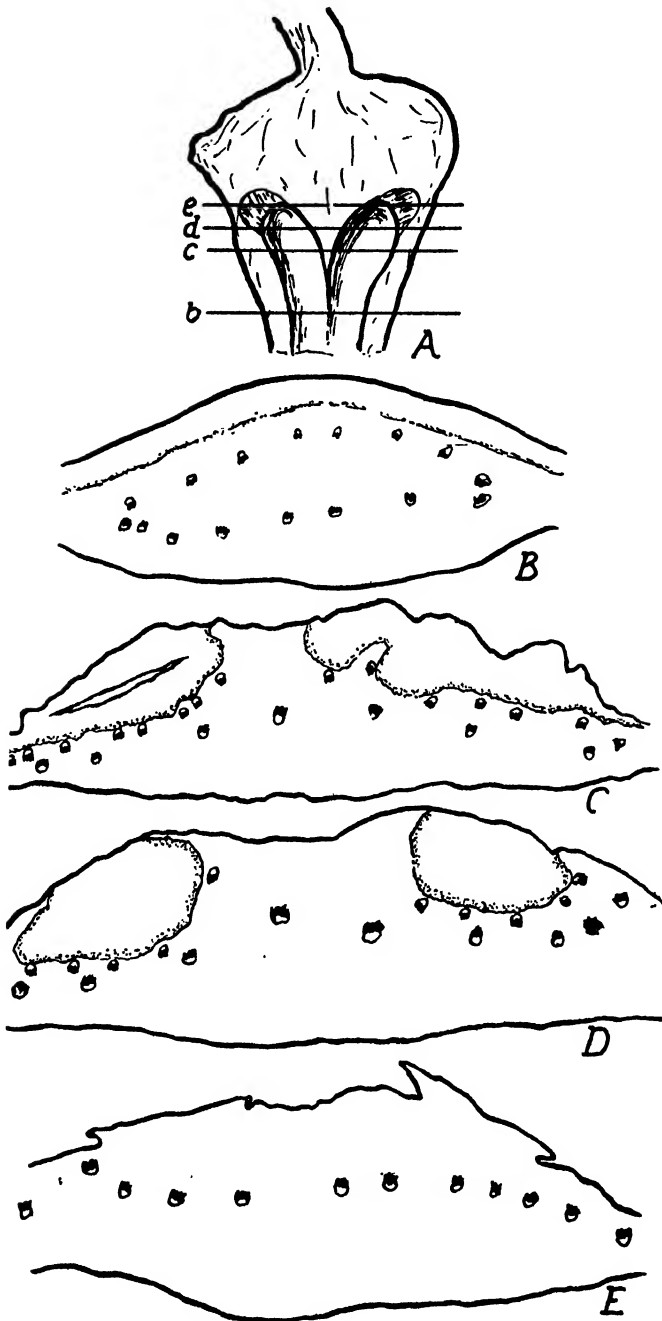
Text-fig. 4 represents serial sections from the base upwards of a scale from the middle of the transition. Here the 'two ligules' are more prominently shown by their swollen and bulged character. They are also placed near to one another, in fact continuous at base, for a short distance.

The 'inverted' supply arises as a branch from the main 'bract' supply near the base of the scale. This divides and re-divides, as does the 'bract supply' bundle, soon forming a uniformly distributed vascular system (Text-fig. 4, B). Also a uniform tissue is apparent on the upper surface of the scale representing the continuous basal portions of the two 'ligules'. Distally this continuous tissue as well as the inverted vascular system become simultaneously divided into two groups (Text-fig. 4, C), the inverted strands following closely the contour of the 'ovular' tissues. Here we also notice a flat cavity which ends blindly. This cavity I interpret as the cavity of an ovule bounded by the integuments, the nucellus having failed to develop.

In sections of a scale with the two 'ligules' more or less 'fused', representing the last stages of the transition, the above description holds good in essential details. An interesting feature is that towards the base of the 'fusing' ovules there are two groups of thick-walled tissue similar in structural details to that of the normal stony layer of the seed. This, in conjunction with the distribution of 'inverted' strands in two groups round the two definitely marked off 'ovular' tissues, in one of which a cavity is also present, gives support to the view that each 'ligule' is associated with an 'ovule'. The external appearance strongly supports this idea (Pl. XXIV, Fig. 1).

The internal anatomy of the normal one-seeded scales is very similar to that of *A. Cookii* (4). Text-fig. 5 shows sections of such a 'scale' at the various levels indicated. The micropyle is very flat, forming a sort of lip on the scale surface (Text-fig. 5, B). In C the nucellar beak is seen engaged with the micropyle. In D a feature to be noticed is the presence of characteristic bulges of thin-walled tissue of elongated tubular cells, on either side, internal to the integument. The manner in which the integument is bulged out to accommodate the tissue is a marked feature of this species and of *A. Cookii*. This tissue may represent the 'inner flesh', and most likely, as suggested by Professor Seward, may be effective in making the mature scales lighter for purposes of dispersal.

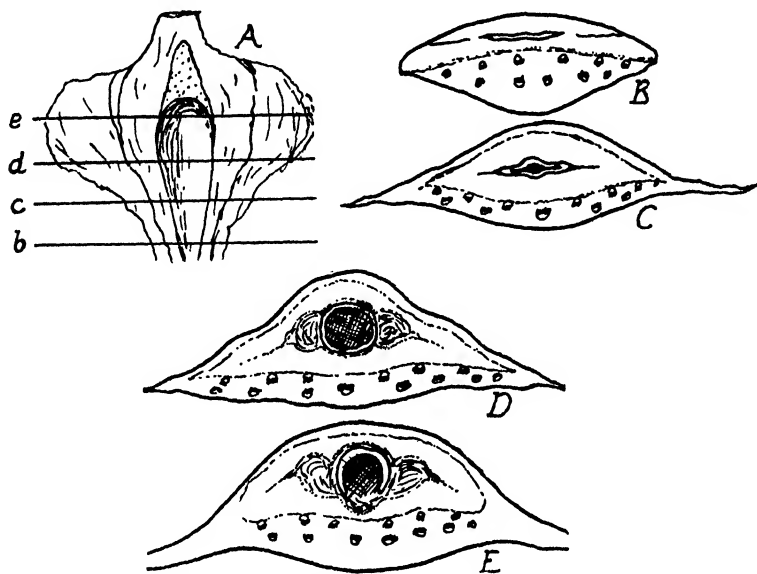
The presence of two 'ligules', and their ultimate 'fusion' into one in the transitional sterile scales, suggest that we may have here the vestiges of two lateral sporangia which have become united into a median solitary one. As regards the vascular supply in these normal scales, a single bundle



TEXT-FIG. 4. A-E. *Araucaria montana*. A. Another cone-scale from the middle of the transition. B, C, D, and E are camera lucida sketches representing sections at levels *b*, *c*, *a*, and *e* respectively.

enters the scale-base from the cone axis, as is usual for the genus as a whole, except *A. Bidwilli*, where the two systems arise independently from the cone-axis cylinder (4). This single strand very near the base gives off an 'inverted' strand. This divides and constitutes the main body of the inverted system whose bundles run in close proximity with the lower and outer face of the stony integument.

Near the base of the ovule some branches are given off into the chalaza, after which the 'inverted' system soon dies out. The 'ligule', at



TEXT-FIG. 5. A-E. *Araucaria montana*. A. A normal 'one-ovuled' cone-scale characteristic of the genus. B, C, D, and E represent semi-diagrammatic sections at levels *b*, *c*, *d*, and *e*.

least the free portion of it, is small and membranous and remains without a supply, as in the majority of species. In *A. Cookii*, however, the 'inverted' system extends a short distance into the free portion of the 'ligule' (Pl. XXIV, Fig. 3).

Araucaria Rulei. The general description given for the transitional scales of *A. montana* in the main holds good for this species. Each of the ovular tissues has its own well-marked 'inverted' supply of bundles. Here, frequently in the transitional cone-scales, the inverted system is seen to be disposed in three groups, one median and two laterals. It is to be noted that the median group is poorly represented, often consisting of a solitary strand. However, two lateral groups are recognizable markedly in the transitional scales of most species. It is worthy of note that a similar state of affairs was observed by Thomson (5) for *Agathis* sp. He suggested that the 'lateral groups' may represent the vestiges of a supply to laterally placed and now missing ovules. It is also very interesting that Hollick

and Jeffrey (6) should have discovered the Cretaceous *Protodammara*, which presumably bore three ovules as indicated by the three scars on the sporophylls.

The normal fertile scale is of the usual type, with a longer 'ligule' than in most species. The three strands of the 'inverted' system run as far as the base, where they turn upwards and anastomose, giving off a small branch into the chalaza from the complex, which soon dies out. The lateral parenchymatous thin-walled tissue mentioned in connexion with *A. montana* as occurring on either side of integument is also recognizable, but is not so prominent

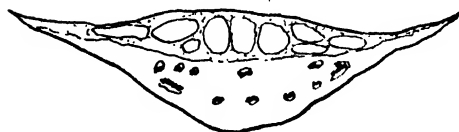


FIG. 6.

TEXT-FIG. 6. *Araucaria Cookii*. Section of a sterile cone-scale from base of cone. Note distribution of inverted strands.

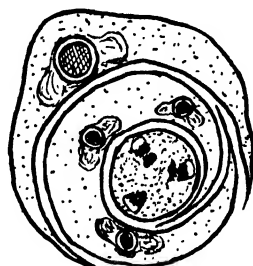


FIG. 7.

TEXT-FIG. 7. *Araucaria Cookii*. Transverse section of apical cone-scales. The cone axis is seen in the middle. Around this is apparently a cone-scale with three ovules. This, however, actually represents three cone-scales grown *en masse* whose apices are still free.

as in that species or *A. Cookii*. For the greater length of the ovule these are present as a flat tissue, but near the middle region of the ovule they present the typical bulged appearance. For the rest my observations confirm those of previous authors (2).

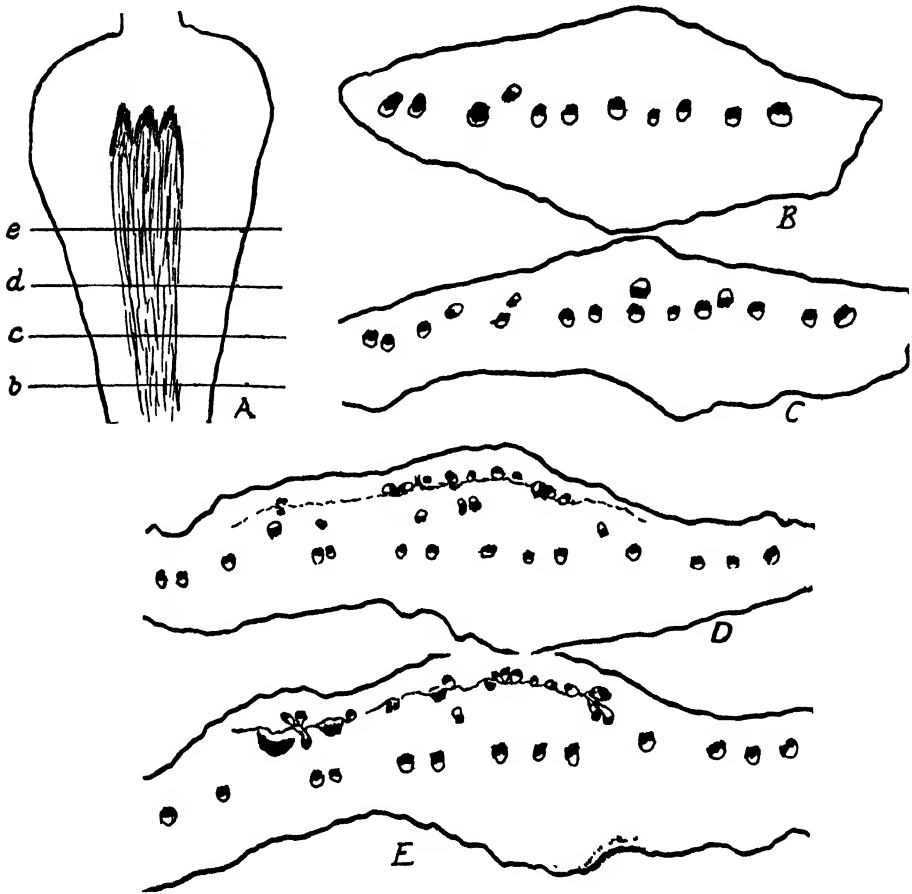
Araucaria Cookii. The internal anatomy of the transitional scales shows certain interesting features. There is towards the upper surface a distinct tissue rich in large 'resin' or mucilage cavities. This probably represents the 'ovular tissues' above mentioned.

The inverted system of strands tends to be disposed in groups (Text-fig. 6), as in the previous species. This suggests that there may be some morphological significance in this grouping of the 'inverted strands'.

The normal fertile scales have been described by Seward and Ford (2), and by Worsdell (4). So far as they go my observations agree with theirs. However, certain hitherto unrecorded features may be described.

The cones, as in *A. montana*, are often fertile to the tip. As in that species, the 4-6 apical scales have grown *en masse* at the base, but are separate at the apex (Text-fig. 7). It is also seen that the cone-axis apex persists, so that apical growth of the shoot through the cone would not be impossible.

The lateral thin-walled tissue on either side of the integument, as mentioned under *A. montana*, is also present here, and is a conspicuous feature of the ripe seed scales (Pl. XXIV, Fig. 4). This tissue was not notice-



TEXT-FIG. 8. A-E. *Araucaria imbricata*. A. A sterile cone-scale from base of cone. B, C, D, and E are camera lucida sketches representing sections at levels b, c, d, and e respectively. Note the distribution of inverted supply strands, and also the presence of a third system of ovular strands.

able in sections of quite young scales. Strasburger (9, Taf. 7, Fig. 68) figures same for *A. Cunninghamii*.

The 'inverted' strands do not soon die out after giving off branches into the ovular base. They run for some distance beyond the ovule, and even into the free portion of the 'ligule' (Pl. XXIV, Fig. 3), ultimately ending in a mass of 'transfusion tracheids'.

Araucaria imbricata. The internal anatomy of the transitional scales is fairly in accord with that presented by other species. Text-fig. 8 represents transverse sections at different levels of the scale shown. A marked

tendency for the 'inverted' supply to be grouped into three regions is evident enough. It will also be seen from the figures that we have here the phenomenon of 'double inversion', giving us a third system of normally orientated strands above the 'inverted system'. This has been noted before in other cases by Thomson (7), and is essentially an ovular supply system. Its persistence on sterile scales is enough indication of its conservative nature.

In sterile scales from a lower region of cone than the previous one, the disposition of the 'inverted' supply in three groups is plainly recognizable, and, as expected, the third system of ovular strands is also present in a less developed form.

In sterile scales from just below the fertile region of the cone, the behaviour of the 'inverted' strands is essentially the same as in the normal fertile scale, there being two main groups laterally placed, which ultimately come together and merge into a confused mass.

In all these scales the 'inverted' strands die out a considerable distance behind the 'ligular' tips. It is curious that the 'inverted' strands sometimes originate in the upper tissues of the scale without any connexion with the vascular supply of the 'bract'. This unconnected origin of 'inverted' strands clearly signifies its independence of the normal scale supply.

The vascular anatomy of the normal fertile scales has been comprehensively dealt with by Seward and Ford (2).

Araucaria Bidwilli. The grouping of the inverted strands is also noticeable in the sterile scales of this species.

The vascular anatomy of the normal fertile scales has been worked out by Worsdell (4). This species is the only one with the 'inverted' supply arising independently direct from the cone-axis cylinder.

It may be worth while now to mention briefly some features of the cone-scales of *Agathis*.

Agathis vitiensis. A single strand enters the fertile scale, and by subdivision forms the normal supply system. This runs for some distance without any 'inverted' bundles. Near the insertion of the ovule most of these normally orientated strands give off an 'inverted' bundle, which together form an upper uniformly spread system. At the insertion of the ovule, 'inverted' strands which are, so to speak, more favourably situated bend upwards and enter the chalaza, anastomosing with one another. The more lateral strands continue beyond this region of ovular insertion, gradually bending upwards, ultimately dying out near the surface of the scale.

Agathis Dammara. The above description applies to this species equally well.

Agathis australis (8). The same essentially as *A. vitiensis*, but here the inverted system consists of only three strands, one median and two

lateral. After giving off a branch into the chalaza, the three soon end near the upper surface of the scale.

Agathis ovata. The vascular anatomy is the same as in *A. australis*.

SUMMARY AND CONCLUSIONS.

From the foregoing description it is evident that there is a transition from 'two-ovuled' to the normal 'one-ovuled' condition, effected in the scales from the base of the cone, and most conspicuously seen in *Araucaria montana* and *A. Rulei*, two species which, curiously enough, are endemic in New Caledonia, a region rich in archaic forms. Whether this transition has any phylogenetic significance or not, it is at present impossible to decide, but the fact that it is fairly constant for at least these two species cannot be neglected. The indication given by other species strongly supports this. I am thus driven to the conclusion that the 'one-ovuled' condition is more probably a 'secondary' and a 'derived feature', rather than a primitive one. It must be noted here that the 'two-ovuled' condition seen in the basal cone-scales of *Araucaria montana* and *A. Rulei* differs essentially from that obtaining in the Abietineae as at present known. In the Abietineae the two ovules are situated on a single axillary 'lamina ovulifera', while in the species of *Araucaria* above mentioned the two 'ovules' are each on a 'lamina ovulifera' (ligule) of its own.

We may suppose that in a scale with three potential ovules the median one alone develops: the scales of *Cunninghamia* and a *Protodammara* may thus represent primitive types, in which all three ovules are developed.

That the ovules, lateral or median, can be suppressed is clearly demonstrated by some of the recent Cupressineae. *Thuja* may be cited as an interesting analogy. Of the usually four pairs of scales in a cone, one fertile pair often has two ovules on each scale, and another fertile pair has one median ovule only on each scale. I do not mean to imply that Cupressineae are a primitive family; they have been thought on valid grounds to be more or less recent in origin. But I am inclined to favour a comparison with the genus *Cunninghamia*, which is very reminiscent of the Araucarians.

The idea of a primitive, presumably three-ovuled scale is supported by the distribution and behaviour of the vascular strands, which tend to be disposed in three groups, the median group being sometimes wanting. Professor Thomson regarded the lateral groups observed by him in *Agathis* sp. (5) as probable remnants of a supply to two lateral missing ovules. I am led to the same conclusion, for this feature is specially prominent in *Araucaria montana* and *A. Rulei*, both New Caledonian species in which, I may say, the 'lateral ovules' are to all intents and purposes no longer missing.

In conclusion my thanks are due to Dr. Boodle for material of *Araucaria imbricata*, to Messrs. C. S. Hanes and T. M. Harris of Cambridge for continuous assistance, and to Professor B. Sahni for his keen interest and criticisms. To Professor Seward I am indebted for his kind supervision and interest throughout the work, and for his valuable criticisms and suggestions.

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EXPLANATION OF PLATE XXIV.

Illustrating Mr. A. K. Mitra's paper on the Occurrence of two Ovules on Araucarian Cone-scales.

Fig. 1. *Araucaria montana*. Cone-scales showing transition from the 'two-ovuled' to the normal 'one-ovuled' scales.

Fig. 2. *Araucaria Bidwilli*. Cone-scales with one or both wings turned in over the median ovule.

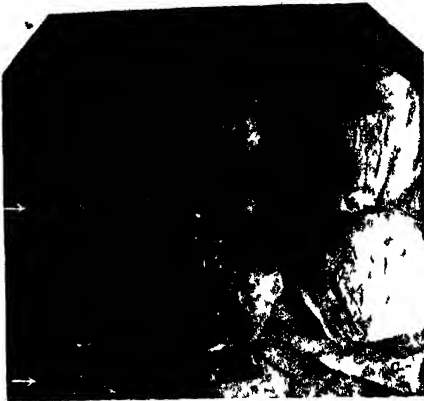
Fig. 3. *Araucaria Cookii*. Transverse section of free portion of ligule: note the vascular strands.

Fig. 4. *Araucaria Cookii*. Transverse section of normal fertile scale showing 'lateral parenchymatous strands'.

Fig. 5. *Araucaria excelsa*. Basal cone-scales with ligules showing bifid and trifid apices.

Fig. 6. *Araucaria montana*. Transverse section of a scale indicated by arrow in Fig. 1.

Fig. 7. *Araucaria Rulei*. Cone-scales showing transition from the 'two-ovuled' to the normal 'one-ovuled' cone-scales.



1. *Araucaria montana*.



2. *Araucaria Bidwilli*.



3. *Araucaria Cookii*



4. *Araucaria Cookii*.



5. *Araucaria excelsa*.



6. *Araucaria montana*.



7. *Araucaria Rulei*.

Studies in the Gramineae¹.

III. Outgrowths of the Reproductive Shoot, and their Bearing on the Significance of Lodicule and Epiblast.

BY

AGNES ARBER, M.A., D.Sc.

With eight Figures in the Text.

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I. INTRODUCTION.

THE spikelet—the unit of the Grass inflorescence—consists of an axis (rachilla) bearing distichous leaves (glumes), the majority of which are axillant to flowers. The structure of the rachilla often diverges widely from that of a typical axis; the pressure exerted by the flowers probably accounts for the curious flattened shape which it sometimes assumes (e. g. 3, Fig. 5, B, p. 455), but even where it is unflattened its anatomy may have

¹ For references to the previous papers in this series, see 3 and 4 in the papers cited, on p. 487.

no pretension to radial symmetry. The form of the axial region of the spikelet is liable to be also complicated by excrescences belonging to two categories: *downward outgrowths* from the median or lateral regions of the leaves, more especially the flowering glumes; and *upward outgrowths* from the tops of the internodes, which show no connexion with any of the leaves. The median downward outgrowths are the commoner, and are referred to by systematists under the names 'callus' (8, 10, 14), 'Schwiele' (10), or 'callous swelling or extension' (14). Despite the frequent allusions to these downward excrescences from the leaves of the spikelets, I know of no account of their anatomy, so I propose to review their structural relations, and to consider a comparison which has been suggested between certain marginal outgrowths and the lodicules of the Grass flower. The upward outgrowths are less frequent and less conspicuous; the examples recorded in the present paper (*Bromus mollis*, L., *B. sterilis*, L., and *Cephalostachyum virgatum*, Kurz) appear to have been hitherto overlooked. Baillon (5), Stapf (14), and others, in describing the inflorescences of *Schizachyrium*, Nees (a section of *Andropogon*, L.), mention 'appendages' ('appendices apicaux') of the 'joints' and 'pedicels' ('articles du rachis'); these may be of the same nature as the upward outgrowths which I am considering in this paper, but I have not been able to satisfy myself on this point, since I have failed to get adequate serial sections of the herbarium material of *Schizachyrium* at my disposal.

Although the upward and downward outgrowths are quite distinct developments, I am treating them side by side in this paper, because in some Grasses it is impossible to understand the construction of the spikelet unless both types of excrescence are taken into consideration. I hope to show that the upward outgrowths of the spikelet axis, which I propose to call *rachilla-flaps*, have an interest of their own, since they may afford a hint as to the meaning of that puzzling little object—the epiblast of the Grass seedling.

Under the heading OBSERVATIONS, descriptions will be found of a few selected cases in which various types of outgrowth can be studied. The DISCUSSION which follows treats of certain theoretical questions suggested by the facts recorded.

I am indebted for material and for other help to the Director and to the Superintendent of the Cambridge Botanic Garden; to the Director and to the Keeper of the Herbarium, the Royal Botanic Gardens, Kew; to the Superintendent of the Royal Botanic Gardens, Sibpur, Calcutta; to Mrs. Agnes Chase of Washington; to Miss G. Lister; and to Professor A. C. Seward, F.R.S. I also wish to express my thanks to Mr. A. W. Gray for help in the study of specimens in the Cambridge Botany School Herbarium. This paper forms part of work carried out with the aid of a grant from the Dixon Fund of the University of London.

2. OBSERVATIONS.

Anthoxanthum. It is well known that the spikelet in the genus *Anthoxanthum* differs somewhat from that of other Gramineae; each spikelet contains a single terminal flower without lodicules, preceded by six

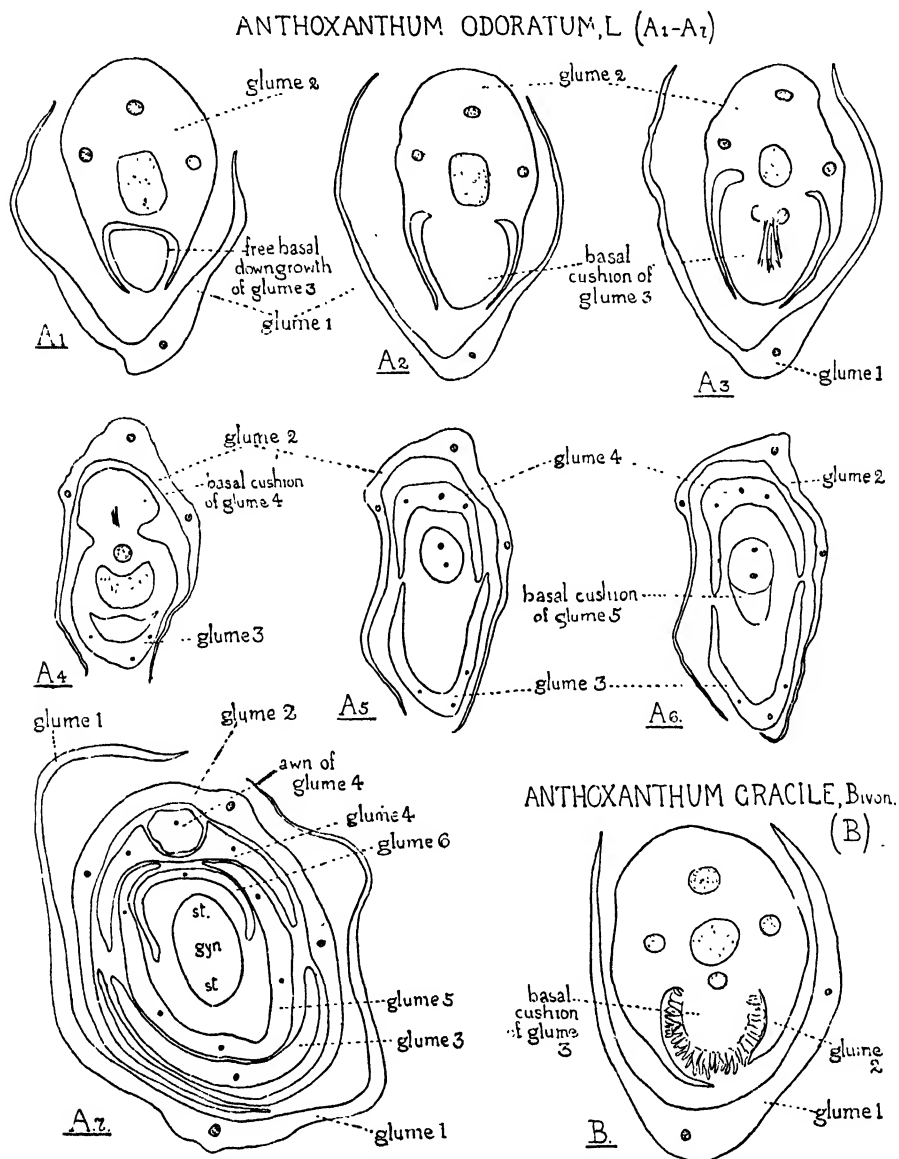


FIG. 1. *Anthoxanthum*. A_1 - A_7 , *A. odoratum*, L., transverse sections from a series from below upwards through a spikelet ($\times 77$); vascular tissue dotted (Lyme Regis, April). B, *A. gracile*, Bivon., transverse section of a spikelet ($\times 77$) passing through glumes 1 and 2, and the basal callosity corresponding to glume 3.

glumes. It is probable that the first and second correspond to the outer empty glumes of more typical Grasses, and the third and fourth to the flowering glumes of two flowers which no longer develop, while the fifth and sixth are the flowering glume and palea of the existing flower. But as the value of these glumes is not the point which concerns us at the moment, I have labelled them in the diagrams with the non-committal names of *glume 1* to *glume 6*. A_1 – A_7 , Fig. 1, p. 475, show selected sections from a transverse series from below upwards through a spikelet of the Vernal Grass, *Anthoxanthum odoratum*, L. In A_1 *glume 1* is already detached, while *glume 2* is still united with the rachilla except at its margins, between which we see a section of the non-vascular, free, basal cushion, which forms the downward prolongation of *glume 3*. In A_2 the attachment of this cushion is seen, but it is still non-vascular; it has a papillose or hairy surface, but this is not shown in the diagram. In A_3 vascular tissue is passing into the glume-cushion. In A_4 the main part of *glume 3* has become free in the median region, and the basal cushion of *glume 4* is appearing on the opposite side. In A_5 *glumes 3* and *4* are both free, and in A_6 the basal cushion corresponding to *glume 5* is making its appearance. In A_7 all six glumes are free and the awn of *glume 4* has come into view; the central mass of tissue, when followed a little higher, is found to give rise to the gynaceum and the two stamens. It will be noted that these diagrams show basal cushions in connexion with *glumes 3*, *4*, and *5*; my series is imperfect and misses the point at which a callosity belonging to the sixth glume might occur, but in a spikelet of the related species, *A. gracile*, Bivon., I have found basal callosities corresponding to each of the *glumes 3*, *4*, *5*, and *6*. The free part of the cushion belonging to *glume 3* of *A. gracile* is shown in Fig. 1, B. The hairy character of its surface is indicated; this is a feature often found in these basal cushions (10).

Ehrharta. The genus *Ehrharta*, which belongs to the same tribe Phalarideae, as *Anthoxanthum*, resembles it in possessing four empty glumes below the flowering glume of the single flower. A–C, Fig. 2, p. 477, show the structure of the spikelet in *Ehrharta panicæ*, Sm. The innermost of the four empty glumes has two remarkable downward wings. Fig. 2, A, shows the wings at the level at which they are free. In B they are united to the rachilla, and in C we come to the normal upper region of *glume 4*, to which they belong. The flowering glume, on the other hand, has a median basal cushion, comparable with those of *Anthoxanthum* (Fig. 2, B). In C the flower itself is reached; it possesses six stamens—a feature unusual among the Gramineae except in the Bamboos. Unlike the flower of *Anthoxanthum*, that of *Ehrharta* is not truly terminal, for the vestigial rachilla can be seen continued above the level of origin of the flower (Fig. 2, C).

Ichnanthus. Another genus in which the glumes show a tendency to

the development of lateral appendages is *Ichnanthus*. A₁–A₃, Fig. 3, represent a series of three transverse sections from below upwards through

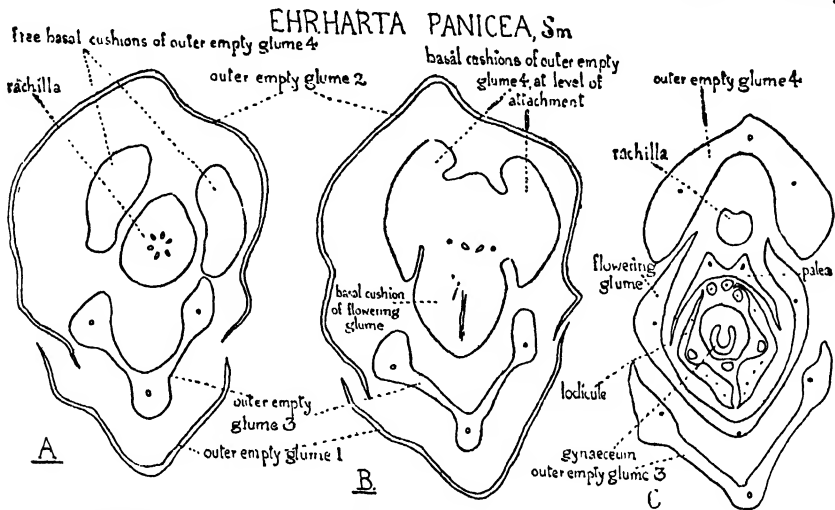


FIG. 2. *Ehrharta panicea*, Sm. (*E. erecta*, Lam.). A–C, sections from a transverse series from below upwards through a spikelet (from Port Elizabeth, Cape Colony; Cambridge Botany School Herbarium); two outer glumes omitted in C ($\times 47$).

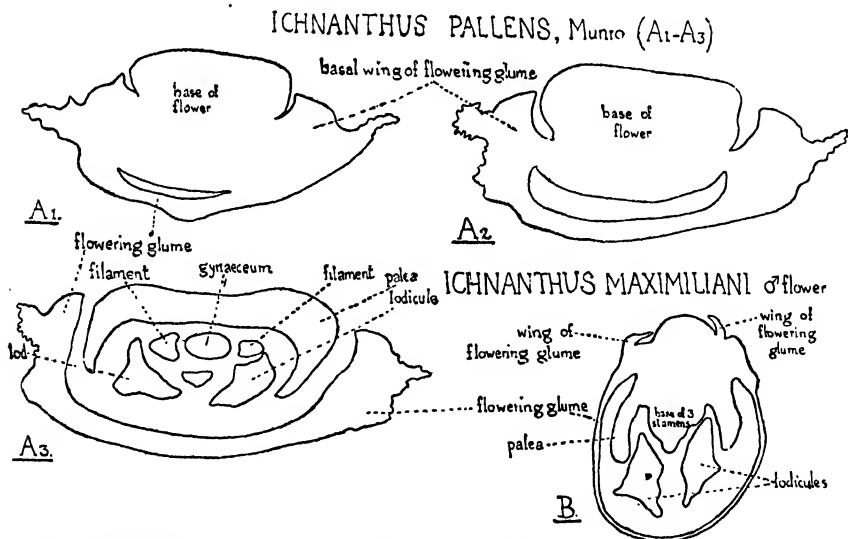


FIG. 3. *Ichnanthus*. A₁–A₃, *I. pallens*, Munro (Khasia; Cambridge Botany School Herbarium); three transverse sections from a series from below upwards through a flower and its glume ($\times 77$). B, *I. Ruprechtii*, Döll, var. *glabratus*, Döll (sheet in Cambridge Herbarium formerly labelled *Ichnanthus Maximiliani*); from Organ Mountains, Brazil; transverse section ($\times 47$) through the base of a male flower to show marginal wings of flowering glume, which is not yet detached except in the median region.

a flower of *Ichnanthus pallens*, Munro, and its flowering glume. A₁ and A₂ show the glume detached in the median region, but, near its edges, still

fused with the base of the flower; the glume-margins form prominent wings. At the level of A_3 the glume and the palea are detached, and the lodicules, filaments, and gynaeceum are seen in section. Fig. 3, B, shows the base of a flower of a plant which is labelled in the diagram *Ichnanthus Maximiliani*, since a sheet in the Cambridge Botany School Herbarium has this name in Bentham's handwriting, but which, since the present paper was in print, has been identified by Mrs. Agnes Chase as *I. Ruprechtii*, Döll, var. *glabratus*, Döll. The diagram B, which represents a male flower cut at the level of the base of the stamens, shows the flowering glume free from the flower-base for the greater part of its width, but fused with it for a short distance at either margin, while the extreme edges are again free and form wings.

Bambusa. I have examined some herbarium material of *Bambusa arundinacea*, Willd., cultivated in the Botanical Garden, Saharanpur, N. India. Serial sections of the spikelet show that the flowering glume of this species has a conspicuous fimbriated basal flap. The structure is not figured here, since I have shown it in 3, Figs. E_1 – E_4 , p. 452. I have not at present noticed a basal glume-cushion in other Bamboos, but I think it would be more easily overlooked in this group than in the other Gramineae.

Bromus. The common British Grass, *Bromus mollis*, L. (sometimes classed as a variety of *B. arvensis*, L.), is well suited to the study of the downward cushion or callus of the flowering glume—an organ exactly comparable with the median glume-cushions of *Anthoxanthum* and *Ehrharta*. But *Bromus mollis* shows, in addition, the upward rachilla-flap referred to in the introduction. So I propose to describe the spikelet in detail and to record—though somewhat diagrammatically—the behaviour of the vascular bundles, and their relation to these excrescences. The structure will be best understood by following A–N, Fig. 4, p. 479, which are taken from a series from below upwards through a spikelet, including the first two flowers and the flowering glume of the third. Fig. 4, A, is a transverse section near the base of the spikelet; the first of the outer empty glumes is omitted, but the second is shown. The most prominent feature of the rachilla is the basal cushion of the first flowering glume. Of the three rachilla bundles, *a* and *b* remain in the rachilla, and *c* passes into the base of the flowering glume, dividing into three; it supplies the palea and all the flower parts. By the time the level B is reached, *a* and *b* have each contributed to a bundle (*a* + *b*) lying between them, and have also given off two bundles, *a'* and *b'*, on the side towards the future flower. In the next diagram, C, the flowering glume is beginning to detach, and the three bundles, *a'*, *b'*, and (*a* + *b*), have taken up the position they will occupy in the succeeding segment of the rachilla, while *a* and *b* are passing off into the margins of the flowering glume. It will be noticed that on the side of the section opposite to the median bundle of flowering glume 1, there is an arc of non-

vascular tissue which I have labelled *rachilla-flap*; it is sharply differentiated from the rest of the section by the large size of the elements of which it is

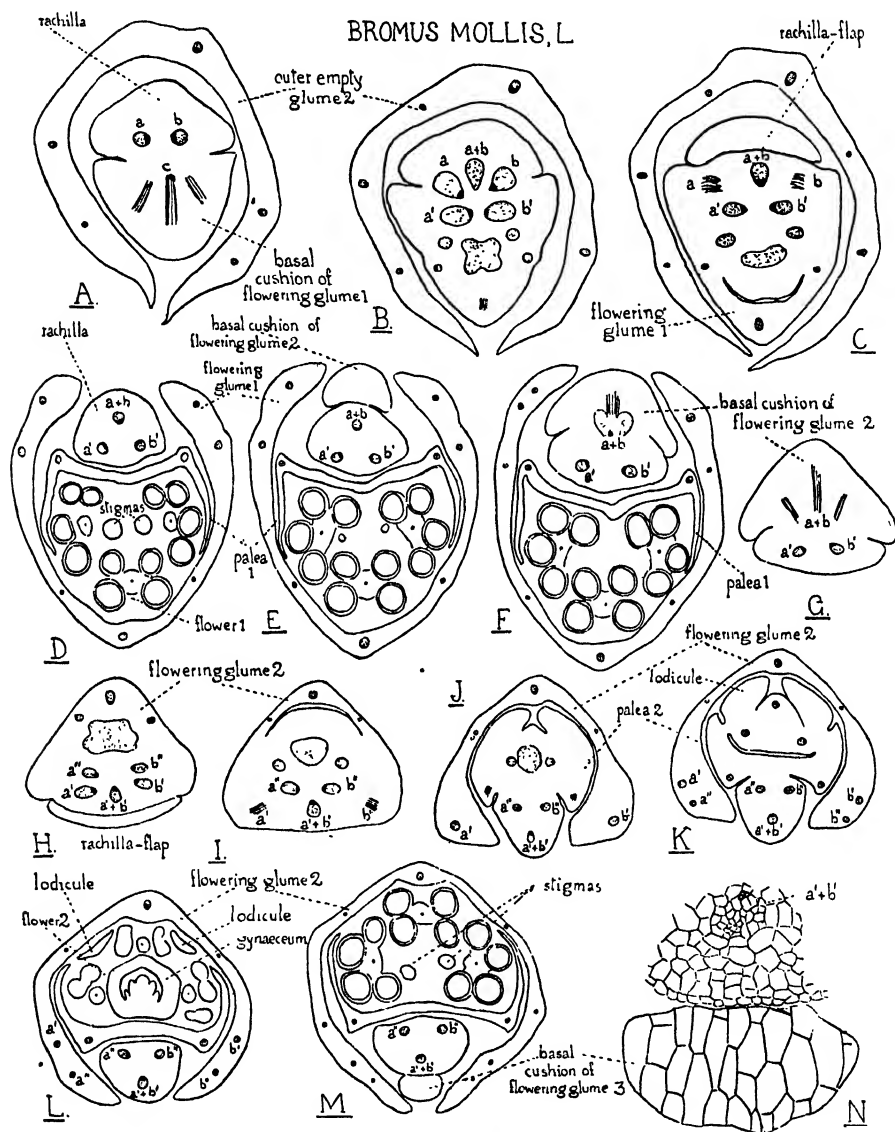


FIG. 4. *Bromus mollis*, L. A-N, sections from a series from below upwards through a spikelet ($\times 47$, except N, which is $\times 193$); vascular bundles treated somewhat diagrammatically. For description, see pp. 480-2.

composed. The rachilla-flap forms the upward termination of the axial tissue of the internode below *flowering glume* 1. In D this flap has disappeared and the rachilla includes the three bundles *a'*, *b'*, and (*a* + *b*), while the flower whose base is shown in C is here cut through the three

stamens and paired stigmas. At the slightly higher level reached in E, we get the first indication of the second flowering glume—the basal cushion (non-vascular at this level) shown to the north; this is a conspicuous object, as it is of different texture to the rachilla and consists of larger cells. In F and G the cushion is expanding in size and the bundle ($a+b$) is passing into it and dividing like bundle c in A. The division of the strands a' and b' follows the same course as in the corresponding bundles below *flower 1*, and five bundles are produced— a' , a'' , ($a'+b'$), b' , and b'' . It will be seen in H, where these bundles can be distinguished, that the 'cushion', from being the small object shown in E, has now come to occupy almost the whole of the section, leaving only a very small non-vascular arc to the south, which

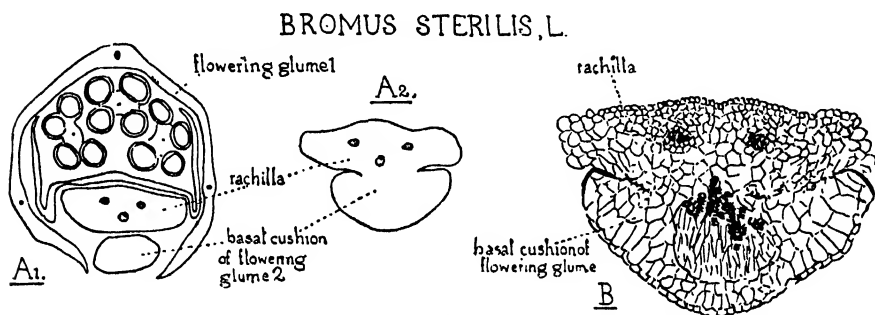


FIG. 5. *Bromus sterilis*, L. A₁ and A₂, transverse sections ($\times 47$) from a series from below upwards through a spikelet. A₁ shows *flowering glume 1* (not the lowest of the spikelet) enclosing a flower cut at the level of the anthers. The rachilla has three bundles, and to the south we see the basal cushion of the next flowering glume, which at this level is free from the rachilla. In A₂, which is a little higher, the cushion is attached to the rachilla. B, transverse section ($\times 77$) of another flower from the same spikelet, to show the third bundle of the rachilla entering the basal cushion of a flowering glume and beginning to divide into three.

forms an upward continuation of the rachilla tissue of the internode below. The bundles a'' , b'' , and ($a'+b'$) supply the next internode of the rachilla, while a' and b' pass into the margins of *flowering glume 2*, and then each divide into two (a' and a'' , b' and b'' , in K). In M we see the first indication of another basal cushion—that of *flowering glume 3*—and thus the history repeats itself. N shows a small part of M more highly magnified, including the basal cushion, which is cut tangentially through its lower epidermis.

Fig. 5 shows for comparison the cushion of the flowering glume of *Bromus sterilis*, L. In Fig. 5, A₁, the downgrowth of *flowering glume 2* is quite free. In A₂, which is cut at a slightly higher level, it is fused with the rachilla, but is still non-vascular. Fig. 5, B, which is cut from another flower, shows the third bundle of the rachilla entering the basal cushion of a flowering glume and beginning to divide into three. *Bromus sterilis* also develops rachilla-flaps corresponding to those of *B. mollis*.

Cephalostachyum. As an example of a plant in which the rachilla-flap is conspicuously developed, we may take a certain Burmese Bamboo,

Cephalostachyum virgatum, Kurz. The illustrations are from material cultivated in the Calcutta Botanic Garden: an account has already been

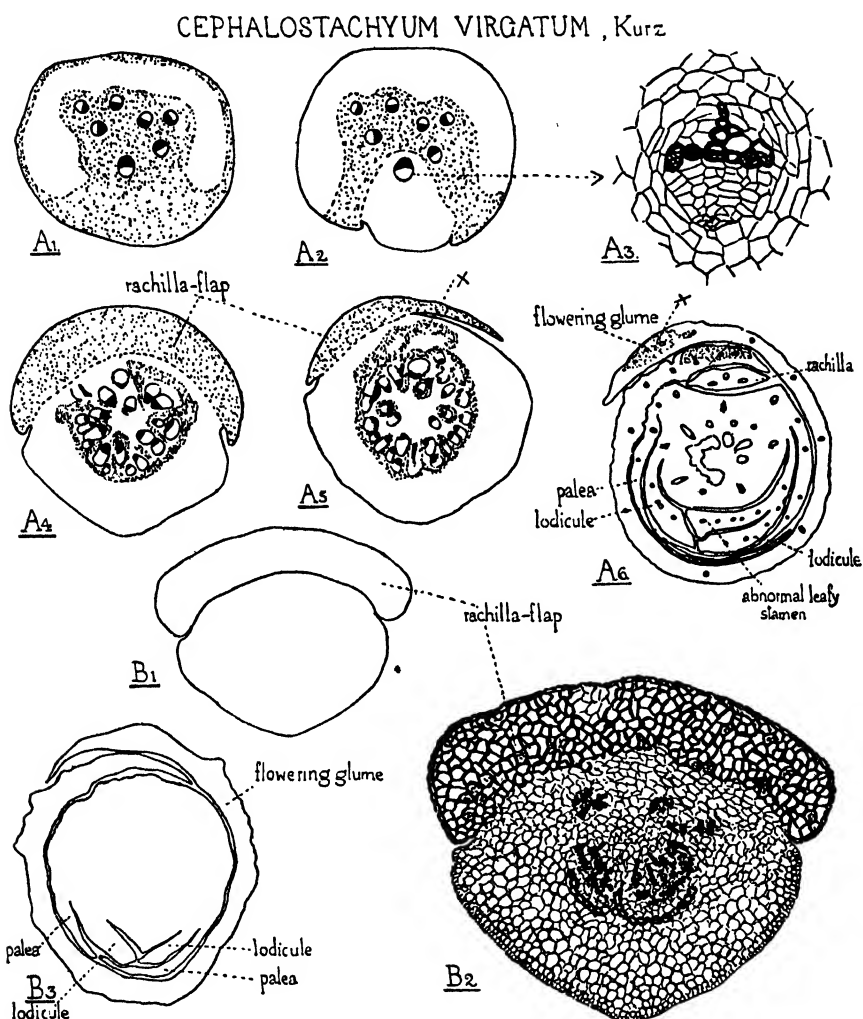


FIG. 6. *Cephalostachyum virgatum*, Kurz (from the Calcutta Botanic Garden). A₁-A₆, transverse sections from a series from below upwards through a spikelet ($\times 47$ except A₃, representing the largest bundle in A₂, which is $\times 318$). B₁-B₃, transverse sections between the two flowers of another spikelet (B₁ and B₂, $\times 47$; B₃, $\times 77$). The axis and rachilla-flap are shown in B₁, and on a larger scale in B₂; the flowering glume and base of the flower are reached at a slightly higher level in B₃.

given of some peculiarities of the flower (4), but the description of the rachilla-flap was postponed to the present paper.

A₁-A₆, Fig. 6, are transverse sections from a series from below upwards through a spikelet. A₁ represents the rachilla below the flower, with the bundles enveloped in fibrous tissue. At a slightly higher level (A₂)

there is a differentiation in the form and structure of the rachilla—the sclerosis has retreated, leaving a non-sclerized patch to the south, whose margins are marked externally by grooves. In A_4 the grooves have travelled farther apart, and they now form the southerly limit of a crescent of non-vascular tissue—the rachilla-flap. The bundles are all left behind in the southerly patch which was first differentiated in A_2 , and which subsequently spread. There is a shell of fibrous tissue round the whole set of bundles, as well as partial fibrous sheaths round the individual bundles. A little higher up, we see that the rachilla-flap becomes free for a minute distance at its apex (A_6); it dies out completely between A_6 and A_8 , at a level at which the next flowering glume is not yet detached, and at which the palea, and the flower to which it corresponds, are still undifferentiated. It will be realized from inspection of the series A_1 – A_6 , that the rachilla-flap may almost be described as the non-vascular upward lip of an oblique cupule, which at its base, on the side opposite the flap, just closes round the rachilla. The detailed structure of the flap, and the contrast between its tissues and the rest of the rachilla, can best be seen in Fig. 6, B_2 . Many of its cells are empty and sclerized with pitted walls; in this section one small patch remains thin-walled.

3. DISCUSSION.

(i) *The Glume-appendages of Ichnanthus and Ehrharta, and the Question of their Relation to the Lodicules.*

The glume-wings of *Ichnanthus* (pp. 476–8, and Fig. 3, p. 477), remarkable as they are, are not without parallel among Monocotyledonous leaves. The section near the base of a flowering glume in Fig. 3, B, p. 477, which shows this member free in the median region, but attached laterally and then free again at the extreme margins, may be compared with the scale-leaf *l. 1* at the base of the lateral bud of *Pistia Stratiotes*, which I have figured in a previous paper (1, p. 100, Fig. 3).

The basal marginal cushions occurring in connexion with the fourth empty glume of *Ehrharta panicea*, Sm., are more distinct than are those of *Ichnanthus* from the glume to which they belong; they are seen as detached entities in Fig. 2, A, p. 477. Eichler (9) emphasizes the resemblance between the glume-wings of these two genera and the lodicules of *Oryza*, which are fused marginally with the base of the palea; he seems to have regarded this resemblance as evidence in favour of Döll's view that certain lodicules are of a stipular nature. In order to find out how far this comparison holds good, I have examined the spikelets of the Rice as well as those of *Ichnanthus* and *Ehrharta*. Fig. 7, A, shows the external appearance of the closely folded palea of *Oryza sativa*, L., with the two lodicules attached basally to its margins. Transverse sections, however, reveal the fact that the lodicules, even in the region in which they are fused to the palea, retain their

individuality, and have an elaborate though delicate vascular system, quite distinct from that of the palea (Fig. 7, B₁). In B₂ the lodicules are cut at a level at which they are free from the palea. The comparison between Fig. 2, A-C, p. 477, and Fig. 7, B₁ and B₂, seems to show little community of character between the glume of *Ehrharta*, with its basal cushions, and the complex formed by the palea and lodicules of *Oryza*, except that in each case we are dealing with a median and two lateral structures, which are connected in the basal region. But small stress can

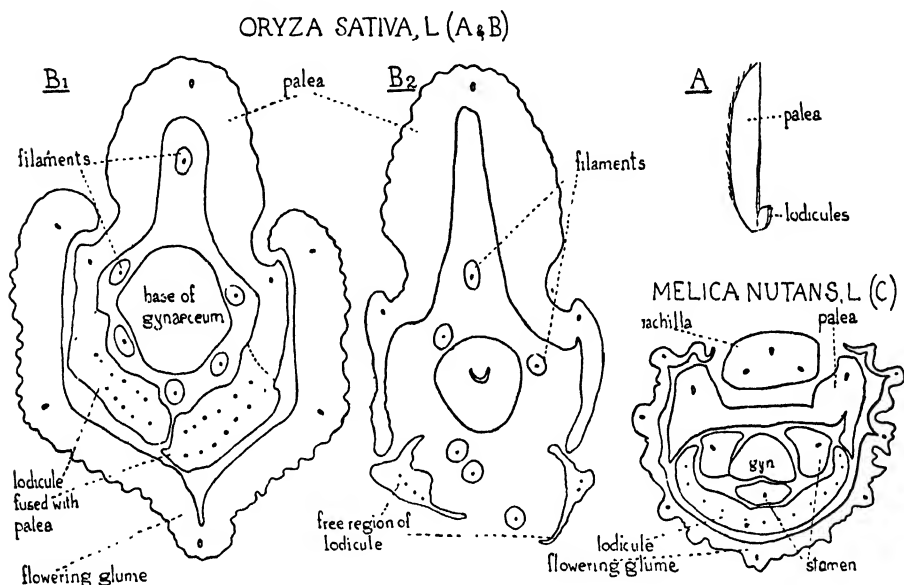


FIG. 7. A and B, *Oryza sativa*, L. A, external view of the folded palea to which the two lodicules are attached at the base (enlarged). B₁ and B₂, two sections from a transverse series from below upwards through a flower ($\times 41$) passing through the filaments of the six stamens. The lodicules are fused with the palea in B₁ and free in B₂. C, *Melica nutans*, L., transverse sections through a flower showing the single anterior lodicule ($\times 41$).

be laid on such connexions, since marginal fusions of organs which are morphologically distinct are not uncommon in the closely packed conditions under which the parts of the Grass flower develop. In various genera the stamen filaments are united (e.g., *Gigantochloa*, 3, Fig. 4, A₁ and D₂, p. 454, and *Oxytenanthera*, 3, Fig. 6, A₁ and B₂, p. 456), while in *Melica* there is a single anterior organ which it seems reasonable to regard as two lodicules connected by their front margins (Fig. 7, C).

On more general grounds there is also an argument of some weight against the theory that the lodicules of *Oryza* are appendages of the palea, and hence against the comparison of these organs with the glume-wings of *Ehrharta* and *Ichnanthus*. If we compare the lodicules of *Oryza* with the two front lodicules of a Bamboo such as *B. nutans*, Wall. (3, p. 450, Fig. 2, A₁ and A₂), we cannot fail to be struck by their close resemblance.

But if we are to call the *front* lodicules of the Bamboo the stipules of the palea, we are driven to suppose that the *back* lodicule (i.e., Fig. 2, A_2 and A_3) is of a different morphological nature. It is true that it diverges somewhat in form and structure from the other two, but the differences seem adequately accounted for by the conditions of pressure under which the organs at the back of the flower develop; and a study of a number of Bamboos inclines me to think that it is highly artificial to place the third lodicule in a different category from the other two. In a previous paper (4) I have recorded some teratological evidence which seems to favour the view that the lodicules represent an inner whorl of perianth members.

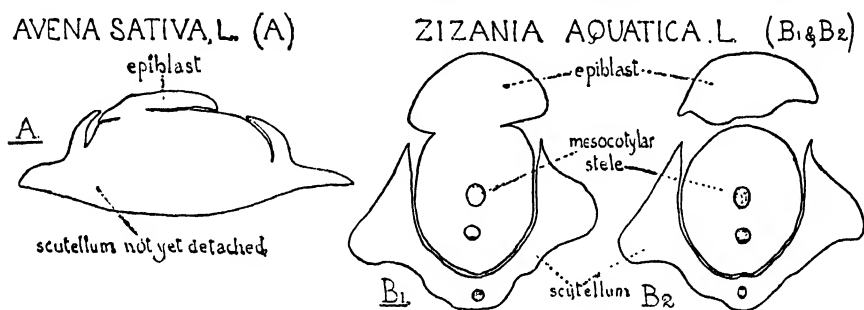


FIG. 8. Epiblasts for comparison with the rachilla-flap. A. *Avena sativa*, L. Transverse sections of the embryo from a seed, cut at a level at which the scutellum is still attached, and the epiblast just freeing itself ($\times 23$); vascular tissue omitted. B_1 and B_2 , *Zizania aquatica*, L. Two transverse sections from a series from below upwards through the attachment of scutellum and epiblast in a seedling ($\times 23$); in B_1 the scutellum is free and the epiblast still attached, while in B_2 both are free.

(ii) *The Comparison of the Rachilla-flap and the Epiblast.*

It will be recalled that in the seedling of certain Grasses a small non-vascular upward tongue arises from the base of the mesocotyl on the side opposite to the scutellum. The most obvious interpretation of this structure is that it represents a second cotyledon, a view which has been maintained in recent years by Bugnon (6). Worsdell (15), on the other hand, has supported a theory first propounded by Čelakovský (7), according to which the epiblast represents the fused auricles of the cotyledon. In a paper published in 1923 (2) I was disposed to accept the latter idea, but the observations recorded in the preceding pages have inclined me to a different view, and I now think that the rachilla-flap may prove a useful term of comparison for the epiblast. If this comparison prove valid, it will harmonize best with the view expressed in 1915 in a joint paper with the late Ethel Sargent (12); in this paper we suggested that the epiblast was merely an outgrowth 'from cotyledon or axis, or both, and of little morphological importance'.

The transverse sections in Fig. 8 show the epiblast of *Avena sativa*, L., and of *Zizania aquatica*, L. That of *Avena* (Fig. 8, A) is represented at a level at which the scutellum is still attached, with the epiblast

just becoming free, while that of *Zizania* is shown below and also above the level of detachment. It will be seen that the epiblast in these two Grasses occupies a position in relation to the scutellum which is not altogether unlike that of the rachilla-flap in relation to the opposite flowering glume. The epiblast and rachilla-flap also resemble one another in their non-vascular character. It must be admitted, on the other hand, that there is great difference in scale between the two structures—the free part of the rachilla-flap being of microscopic dimensions in the cases which I have figured. But it may be recalled that in the West African Grass *Urelytrum squarrosus*, Hæck., the ‘appendages of the joints’, which may possibly be comparable with our rachilla-flaps though belonging to axes of a different order, reach a length of over 4 mm. (14). The most important difference between the epiblast of *Zizania* and *Avena* and the rachilla-flap is however a question of structure, not of scale; it is that these epiblasts lack the cupular character which is shown very clearly by the rachilla-flap of *Cephalostachyum*, and of which there is a slight indication in *Bromus*. It would, indeed, be unsafe to push the comparison between these structures to any great length, but I think that there is, at least, justification for saying that the rachilla-flap and epiblast fall into the same category. They probably both owe their existence to the tendency to the development of non-vascular excrescences which seems to be a natural characteristic of the Gramineae. This tendency, which affects all parts of the plant body—shoot and root alike—is exhibited, for instance, in the various basal outgrowths of the glumes which have been described in this paper; in the ‘lodicule cushions’ of *Schizostachyum brachycladum*, Kurz (3, p. 460 and Fig. 9, A, p. 462); in the pulvini at the angles between the main axis of the inflorescence and its branches in such Grasses as *Dactylis glomerata*, L. (11, Bd. I, Abt. 2, pp. 97–9); and in the coleorhiza of the seedling.

(iii) *The Rachilla-flap and the Leaf-skin Theory.*

The interesting conception which E. R. Saunders has called ‘The Leaf-skin Theory of the Stem’ (13) has, I think, been of great value in breaking down the conventional view of stem and leaf, and in many cases it undoubtedly provides a useful mode of visualizing the surface features of the stem and leaf complex. It does not seem, however, to be applicable to all shoots. It is for instance difficult, if not impossible, to describe the rachilla-flap considered in the present paper in terms of the leaf-skin. In the case of *Bromus mollis*, L., immediately above the top of the flap we come to the margins of the next flowering glume, which at their extreme base just meet round the rachilla. But the tissue of the rachilla-flap is utterly unlike that of the glume and shows no continuity with it. This point comes out even more strongly in *Cephalostachyum virgatum*, Kurz. Here the margins of the flowering glume, instead of barely fusing, as in *Bromus mollis*, overlap

to a considerable extent, and in the example drawn in Fig. 6, A_6 , p. 481, they are decidedly fibrous. It is of interest to find, on examining A_5 (which is from a section cut at a slightly lower level), that the fibres marked with a cross, which lie in the main body of the axis and not in the flap, are continuous above with the fibres marked with a cross in A_6 , which belong to the outer margin of the flowering glume. These fibres thus serve to locate the glume tissue before it leaves the axis, and to emphasize its lack of connexion with the rachilla-flap.

(iv) *The Stem as a Morphological Unit.*

If one examines the series through a spikelet of *Bromus mollis*, L., drawn in Fig. 4, p. 479, it requires some sophistry to cling to the idea of the rachilla as a continuous individual organ; it may almost be said to be dissolved and reconstructed at each node. At the level of Fig. 4, H, for instance, if one insists on recognizing the rachilla as an independent entity, one is driven to use the name for the three bundles a'' , b'' , and $(a' + b')$, and for a certain amount of tissue round them, whose limits are scarcely possible to define, since the bundles a' and b' , which are destined for *flowering glume 2*, must be excluded. This discontinuity in the rachilla is also seen in *Cephalostachyum virgatum*, Kurz, on comparing Fig. 6, A_5 and A_6 , p. 481. If 'the axis' is to be treated as a definite morphological unit, we must identify the whole of the large, richly vascular transverse section in A_5 with the small, three-bundled organ marked *rachilla* in A_6 , a section which is very slightly higher in the series, being cut at a level at which the flowering glume has become free and the palea and floral parts have begun to separate. Indeed, an examination of the Grass rachilla tends to shake one's faith in the stem as a morphological unit. I cannot here discuss the questions thus raised regarding the nature of the shoot in the flowering plants, but I hope to offer a study of these difficult problems in a later paper.

4. SUMMARY.

1. *Observations.*

The following structures are studied in the present paper:—The *median basal outgrowths* of the glumes of *Anthoxanthum* (pp. 475 and 476 and Fig. 1, p. 475), *Ehrharta* (p. 476 and Fig. 2, B, p. 477), and *Bromus* (pp. 478–80 and Figs. 4 and 5, pp. 479 and 480); the *lateral basal outgrowths* of the glumes of *Ehrharta* (p. 476 and Fig. 2, p. 477) and *Ichnanthus* (pp. 476–8 and Fig. 3, p. 477); the *upward outgrowths* from the top of the internodes of the spikelet axis (*rachilla-flaps*) in *Bromus* (pp. 478–80 and Fig. 4, p. 479) and *Cephalostachyum* (pp. 480–2 and Fig. 6, p. 481).

2. Discussion.

(i) The suggestion that the glume-appendages of *Ehrharta* and *Ichnanthus* may be compared with the lodicules of *Oryza*, is considered, and an attempt is made to show that this comparison is untenable (pp. 482-4 and Fig. 7, p. 483) and that the stipular theory of the lodicules cannot be maintained.

(ii) Certain resemblances between the rachilla-flap and the epiblast are pointed out, and it is suggested that these two structures are of the same nature, in so far as they are both outgrowths of the tissues of the axis and are not comparable with leaves (pp. 484 and 485).

(iii) The rachilla-flap is considered in its relations as a part of a shoot (the spikelet), and it is concluded that it does not lend itself to description in terms of the leaf-skin theory of E. R. Saunders (pp. 485 and 486).

(iv) Attention is drawn to the spikelet as an example of a shoot, and it is shown that the behaviour of the rachilla is calculated to raise doubts as to the validity of the orthodox view of the axis as a morphological unit (p. 486). It is hoped to pursue this subject farther in a later paper.

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Germination in *Lachnea cretea*.

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With three Figures in the Text.

IN 1922 Dodge (1) described a small, discomycetous fungus which he identified as *Lachnea abundans*, Karst., *Lachnea cretea*, (Cooke) Phil., being regarded as a possible synonym, and noted the crops of botryose conidia developed from its mycelium. He called attention to the resemblance of the ascocarps to those investigated by one of us (2) in 1913, but was deterred from accepting the two fungi as identical owing to the lack of information as to the conidial stages of *L. cretea*.

In the study of the latter species completed in 1913 attention was concentrated on the archicarp and its products, and while material was grown free from any inconvenient infection, no attempt was made to work with single spore-cultures. The determination of the relation of the ascocarps to the conidia sometimes noticed on recently infected plates was not undertaken.

Cultures of the species in question had, however, been kept in the laboratory since 1913, and, in view of the points raised by Dodge, a study of growth and germination was undertaken. There was no difficulty in obtaining abundance both of conidia and ascocarps by mass infection from tubes put up in 1923, and later from single spores.

The mycelium grew well on cow-dung agar and on a number of synthetic media, the best for our purpose being Barnes's medium (3) (0.1 per cent. K_3PO_4 , 0.1 per cent. NH_4NO_3 , 0.1 per cent. K_3NO_2) made up with 0.1 per cent. glucose. Another medium, 'M', made up with 0.1 per cent. each of K_3PO_4 , NH_4NO_3 , and $MgSO_4$, was also of some importance, as it gave good mycelial growth and numerous ascocarps, but no conidia, or only a few round the edge; it was particularly useful in providing ascocarps free from conidia.

The conidiophores arise singly on the mycelium and branch in a dichotomous manner (Fig. 1), their ends become swollen, sterigmata are put out, and numerous conidia are formed. Our material differs from that figured by Dodge chiefly in the much larger number of transverse septa in the filaments of the latter. So far as it is possible to judge, the ascocarps

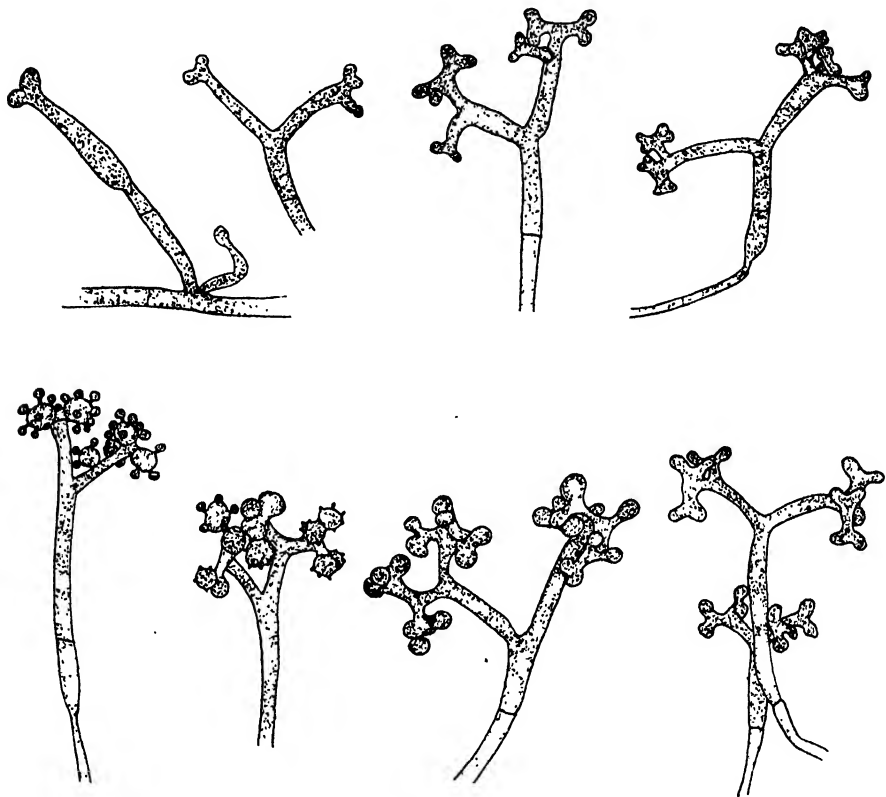


FIG. 1. *Lachnea cretea*. Stages in the development of the conidia. $\times 280$.

differ only in colour, Dodge describing an ochraceous tint which we have not observed except in old cultures infected with bacteria. Doubtless the culture medium is important in such respects.

Cultures were inoculated with a single conidium by the dilution method ; germination took place at ordinary temperatures without delay ; an anastomosing mycelium was obtained (Fig. 2) ; conidia appeared five to seven days after inoculation, and ascocarps in seven to twelve days. Material was grown at temperatures between 18° and 25° C., the ordinary temperature of the laboratory being between 18° and 22° C.

Single ascospores for inoculation were picked off the lid of a Petri dish containing a good culture by means of a fine capillary tube, trans-

ferred to plates of media similar to those on which the conidia were grown, and kept at ordinary laboratory temperatures. In the first twelve plates prepared in this way not a single germination was observed.

Three plates of Barnes's medium + 0.1 per cent. glucose were then prepared; one was inoculated with a conidium and the others with ascospores. All three were placed at 70° C. for a quarter of an hour, a procedure stated by Dodge to ensure germination. They were cooled off at 38° C. for an hour and then placed in the open laboratory at 20° C. In two days the plate inoculated with a conidium showed growth, but no germination took place in the others; nor were repetitions of the experiment more successful. Ascospores similarly failed to germinate after fifteen minutes at 60° and 50° C.; in case the time of year had a bearing on germination, these experi-

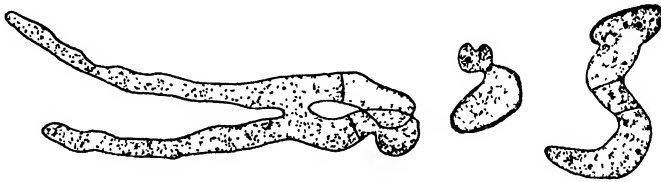


FIG. 2. *Lachnea cretea*. a. Two conidia with germ-tubes between which anastomosis has taken place. b. Germinating ascospores. $\times 420$.

ments, originally carried out in February and March, 1926, were repeated in June, but still without result.

On July 13, however, six plates were infected with ascospores, subjected, for fifteen minutes, three to a temperature of 70° C., and three to a temperature of 65° C., cooled for eighteen hours at 38° C., and set on a window sill in the sun. Two days later germinations were found in each of the six plates, and germinating ascospores were also observed lying near the ascocarps in old, untreated cultures which were standing in the same window. The weather had been very hot, the sun shining directly upon the cultures, which had thus been subjected to a temperature, so far as it is possible to judge the temperature of strong insolation, of about 48.5° C., as well as to bright light. Plates inoculated with ascospores were accordingly incubated, both in light and in darkness, for three hours at 48.5° C., and afterwards set in a good light at a temperature of 18° to 20° C. In twenty-four hours ascospores had germinated in all the plates, showing that the heat rather than the light to which the spores had been submitted in bright sunshine was responsible for their growth.

In germination the ascospore swells to about twice its size, and puts out one or more germ-tubes (Figs. 2, b, and 3), which readily anastomose and in due course give rise to conidia and ascocarps. On suitable media conidia are occasionally produced from the germ-tube itself.

An attempt was next made to determine the limits of temperature inducing germination and the limits of time for which the spores must be submitted to each. On plates incubated at 55°C . for three hours and afterwards placed in the open laboratory no germinations were found for three days, and then only two spores germinated out of eighteen, indicating that the upper limit of temperature and time had been approached. On plates incubated for three hours at 52°C ., but otherwise similarly treated, no

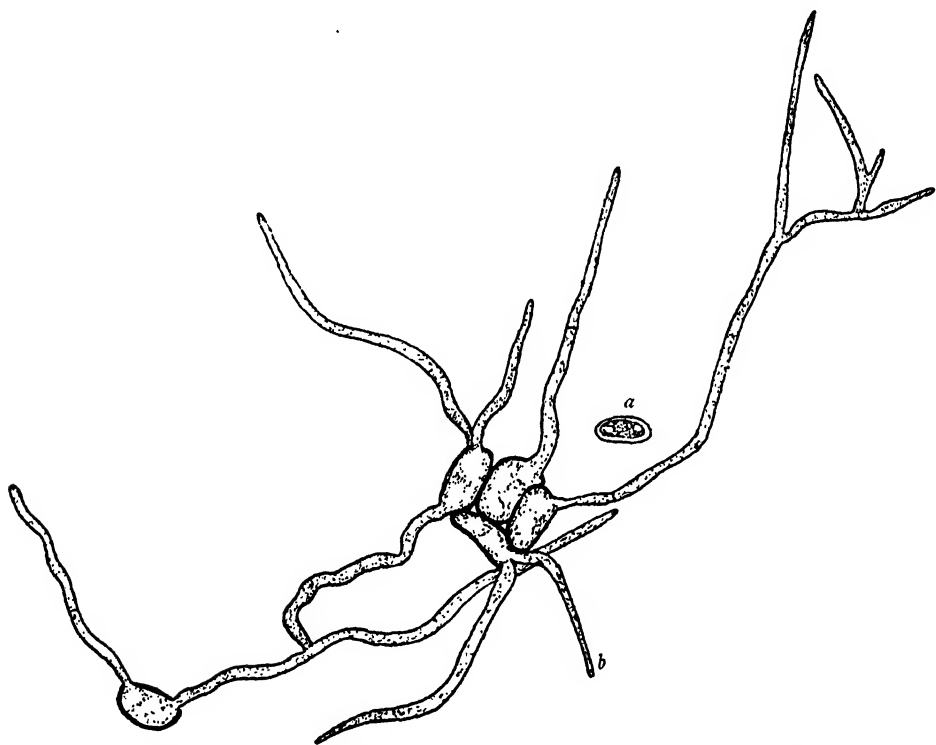


FIG. 3. *Lachnea cretea*. *a*. Ascospore when first shed. *b*. Group of ascospores germinating in sunlight on a stale medium. $\times 420$.

germinations took place for three days, but at the end of five days most of the spores had begun to grow and good mycelium was produced. On plates kept at 42°C . for three hours and then treated as before, no germinations could be discovered, showing that for the spores and time in question the lower limit lay between 48.5° and 42°C . On plates incubated at 55°C . for one and a half hours only, and then placed at laboratory temperatures, germination was delayed but not prevented; three spores out of thirty-four had formed germ-tubes at the end of two days, and several others a couple of days later.

As there seemed indications of a relation between time and temperature, attention was again turned to Dodge's experience that ascospores

germinated at ordinary temperatures after fifteen minutes at 70° C., a method which had hitherto proved unsuccessful in our laboratory. It was recalled that 'ordinary' temperatures were probably higher in Washington than in London, and spores were accordingly placed for fifteen minutes at 70° C. and afterwards left at 38° C. Germination took place in eighteen hours, while no growth could be found in the controls kept at 38° C. throughout. Further experiments showed that fifteen minutes at 60° or 70° C., followed by temperatures between 29° and 42° C., resulted in good growth, but that, even after the preliminary stimulus, temperatures of 25° C. or less were insufficient to induce germination.

Temperatures above 70° C. were also found effective, ascospores germinating readily after exposure for five minutes to 100°, 125°, and even 150° C., followed by incubation at 30° C.

In all cases laboratory temperatures (18° to 22° C.) were adequate for development and for the formation of ascocarps and conidia once growth was fairly started.

Meanwhile the question was raised of the age of the ascospore as a possible factor in germination. The experiments already carried out were reviewed and others undertaken from this standpoint. The spores which had germinated after exposure to strong sunlight (about 48·5° C.) had been grown on a culture on which spores began to be shed some ten days previously; they were therefore at most ten days old, counting from the date of liberation. Spores as young as eight days or less germinated after similar treatment.

Spores of the same age and up to twenty-four days germinated, as described above, if exposed to 55° C. for one and a half hours, to 52° C. for three hours, or, in a few instances, after exposure for three hours to 55° C. They also germinated after exposure to 60° or 70° C. for fifteen minutes, provided that they were afterwards kept at a temperature over 25° C. until the germ-tubes had appeared. 55° C. for fifteen minutes proved ineffective.

Ascospores six weeks old germinated under the above-mentioned conditions, and also at laboratory temperatures after exposure to 55° C. for only one hour.

Ascospores seven weeks old germinated at laboratory temperatures after one hour at 48·5° C. and after half an hour at 55° C., but failed to germinate after exposure to 48·5° C. for half an hour. They germinated also, like younger spores, after fifteen minutes at temperatures of 60° C. or more, followed by incubation at 30° C. Controls of the same age, kept at 30° C. throughout the experiment, failed to show germination.

Ascospores seven weeks and two days old germinated somewhat more readily; 48·5° C. for half an hour and 55° C. for a quarter of an hour proved adequate followed by laboratory temperatures, and good germinations were obtained after exposure for five minutes to temperatures as high as 150° C.

followed by incubation at 30° C. Spores left for half an hour at 150° C. and those exposed for five minutes to 190° C. could not be induced to germinate by any subsequent treatment. In controls kept at 30° C. throughout several spores germinated; this was not the case with any younger spores.

Ascospores eight weeks old germinated after a quarter of an hour at 48.5° C. After exposure to 30° C. for three or six hours, followed by 20° C., there was no germination, but one spore germinated after nine hours at 30° C. followed by 20° C. 25° C. for sixteen hours, followed by 20° C., showed a few spores germinating twenty-four hours after the experiment began, and, among a number exposed to 18° to 20° C. throughout, one spore was found to germinate after considerable delay. This was the first case of germination at ordinary temperatures without preliminary heating; all younger spores had required at least nine hours at 30° C., a short time at 48.5° C., or a few minutes at a temperature between 60° and 150° C.

Ascospores thirteen weeks old gave good mycelium with conidia and ascocarps at 18° C.

The oldest spores available were those from cultures inoculated on the 16th of March, 1923, and kept as stock; they were therefore three and three-quarter years old when used in January, 1927. Ascospores germinated seven days after being placed on agar made up with medium 'M' + 0.1 per cent. glucose and produced conidia and ascocarps at temperatures between 22° and 25° C. They also germinated at laboratory temperatures after two hours at 48.5° C. They failed to germinate, however, when subjected to a temperature of 70° C. for fifteen minutes, or 100° C. for five minutes before being placed at laboratory temperatures.

It appears from these experiments that the ascospore is not ready to germinate at the time it is shed, but undergoes some further process of ripening which takes at least eight weeks. This may be accelerated in spores up to three weeks old by some hours of hot sunshine, after which spores germinate at ordinary temperatures, or artificially by incubation for three hours or longer at 48.5° C., followed by 18° C., or for fifteen minutes at 70° C., or five minutes at temperatures between 100° C. and 150° C., followed by temperatures above 29° C. For older spores progressively less drastic treatment is necessary, spores seven weeks and two days old germinating at 30° C. The higher temperatures which induce germination in young spores will actually inhibit development in those which are already old. It seems evident that chemical changes are in progress in the spore after it has left the ascus, that germination cannot take place till these are accomplished, and that their rate may be accelerated by judicious application of heat.

Conidia, on the other hand, germinate readily as soon as they are shed, but die before the ascospores, since in those three and three-quarter years old no germination could be induced.

The germination of conidia was found to be inhibited by cold; neither

they nor the ascospores would germinate at temperatures below 5° C., but were not killed even by several hours at - 2° C., and germinated when restored to warm conditions.

SUMMARY.

1. Ascocarps of *Lachnea cretea*, (Cooke) Phil., have been obtained from conidia, and conidia from ascospores.

2. The conidia arise on dichotomously branched conidiophores, the ends of which become swollen and covered with sterigmata.

3. The conidia germinate readily at temperatures above 5° C.; germination will not take place at - 2° C., but the conidia are not killed.

4. Ascospores will not germinate at temperatures of 18° or 20° C. till eight weeks after they have left the ascus.

5. Germination was induced in younger spores by some hours of hot sunshine, giving a temperature of about 48.5° C. The same result was obtained artificially by incubation at appropriate temperatures. Light is not necessary for germination.

6. For spores of intermediate age, progressively shorter exposures to high temperatures are adequate, or incubation at progressively lower temperatures.

7. Ascospores three and three-quarter years old germinated readily, but conidia of the same age could not be induced to germinate.

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On the Relation of Soil Temperature to Angular Leaf-spot of Cotton.

BY

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BACTERIAL disease of cotton caused by *Pseudomonas malvacearum*, E. F. Smith, was first discovered in the Sudan by the writer during the season 1922-3, and rapidly assumed serious proportions.

Following on the failure of attempts to control the disease by means of external disinfection, bacteriological studies were undertaken by Major R. G. Archibald, D.S.O. (1), who discovered the parasite within the cotyledonary tissues of the resting seed.

Work on this disease was resumed by the writer in 1926 on physiological lines, in the hope that some light might be thrown on the predisposing causes.

Previous to the observations about to be described, every available meteorological record had been examined without success to find a clue to the incidence of the disease; its distribution in regions varying from desert to that of the wet tropics, and over such a wide range of air temperatures, baffled inquiry. In field and laboratory experiments the apparently erratic behaviour of the parasite also led to numerous investigations without definite results.

Soil conditions were also studied, but an early experiment (Expt. 1, below) showed that while an alkaline soil may, and does, affect the vigour of the seedling plant, it is not the principal factor concerned with the manifestations of the disease, or the favourable development of the causal bacillus.

At the outset, two points should be made clear: firstly, the experiments detailed are only concerned with the seedling stage—the subsequent history of the parasite within the growing and adult cotton plant must be dealt with at a future date; and secondly, that all the temperatures recorded, except where otherwise specifically stated, refer to the soil at a depth of two inches, i. e. the depth at which the seed is planted.

It may be of interest to describe the means taken to demonstrate the presence of the parasite within the seed used.

The seed was gathered from cotton plants showing characteristic symptoms of the disease, and was immersed for twenty minutes in concentrated sulphuric acid. After washing, it was again disinfected in 1/1,000 mercuric chloride and finally washed with sterile distilled water. The treated seed was then sown in large Erlenmeyer flasks in which was a layer of agar containing the usual nutrient salts, and the flasks placed in a north window with the mouths well stoppered with cotton-wool.

After twelve days seedlings in each flask were found to show the characteristic lesions of bacterial disease, and the organism *P. malvacearum* was recovered in pure culture.

SOIL TEMPERATURE EXPERIMENTS.

Experiment I, January 14, 1925. (a) Highly infected Pima seed was sown in tumblers containing 250 grm. of a heavy clay soil, the pH of which was 9.3. Water was added as follows, and the whole series maintained at a temperature of 27°–28° C. by means of an electric lamp.

Tumblers No.	1–6	125 c.c.	Soil liquid mud.
	7–12	100 "	" saturated.
	13–18	75 "	" moist.
	19–24	50 "	" just moist.

The seedlings in each case grew without trace of disease, in spite of the fact that Nos. 1–12 were lanky and weak.

(b) Simultaneously a series of seedlings were grown from the same seed in 4-in. pots under light shade, and watered normally, i. e. sufficiently to keep the soil moist.

Unfortunately the soil temperatures were not taken at this time, but the fact that the minimum air temperature in the shed was 15° C. renders it certain that the temperature of the pots was below 20° C. The seedlings developed slowly, and 14 days after sowing 73 per cent. showed definite bacterial lesions.

Expt. II, July 14, 1926. In this and the following experiments a sample of Ashmouni seed known to be heavily infected with *P. malvacearum* was used. The seeds were untreated in any way and were sown in pots similar to Expt. I (b), but a light loam was used. From this period onwards soil temperatures were included in the observations, along with maximum and minimum and relative humidity determinations.

The average soil temperature at 8 a.m. was 25.5° C.; the maximum recorded was 28.5° C.

Air humidity varied from 50 per cent. at 8 a.m. to 45 per cent. at 2 p.m.

Growth was rapid, and after seventeen days it was found that 95 per cent. of the seedlings showed characteristic lesions.

Expt. III. Suspicion began to centre definitely around the temperature of the soil, and accordingly a similar number of pots (i.e. 50) were sown with five seeds each and enclosed in a cupboard with glass sides, the soil temperature being maintained at 28° – 33° C.

The humidity was naturally higher in the enclosed space and ranged from 75 per cent. to 100 per cent. The resulting seedlings were remarkably healthy in appearance, being very dark green, and seventeen days afterwards showed no signs of disease whatever. Moreover, the cause of heat was withdrawn and the cupboard gradually opened for some fourteen days afterwards without development of disease.

Expt. IV, August 28, 1926. It was noted that the temperature of the soil in boxes was higher than that in pots; accordingly 500 seeds were sown in two series, (a) 250 seeds in pots as before, (b) 250 seeds in boxes, and the two series placed side by side in the shed. The temperatures recorded were as follows :

(a) <i>Pots.</i>	Average soil temperature at 8 a.m.	25.5° C.
	Maximum soil temperature recorded	27.5° C.
(b) <i>Boxes.</i>	Average soil temperature at 8 a.m.	27.5° C.
	Maximum soil temperature recorded	31.9° C.

The air humidity was of course the same in both cases and varied from 45 per cent. to 65 per cent.

After twelve days 96 per cent. of the seedlings in the pots showed signs of infection, whilst only 3 per cent. of the seedlings in the boxes were infected.

Expt. V, August 28, 1926. All the preceding experiments had been conducted in light shade, and, whilst it was realized that the fluctuations in temperature would be greater, it was thought worth while to ascertain whether or not shade exerted any effect. Two series were therefore started : (a) Fifty pots sown with the same variety of the seed and placed in sunshine and wind. The average soil temperature of these was 26.2° C. at 8 a.m., rising to 34° C. at 2 p.m. (b) A similar series, but in the shade. The average soil temperature at 8 a.m. of these was 24.2° C., rising to 25.2° C. at 2 p.m.

These seedlings were examined fourteen days after sowing, with the following results :

Sun series, infection 27.7 per cent.

Shade series, infection 46.7 per cent.

This experiment was maintained for a month longer, by which time the wind had changed to the north, with a consequent drop in humidity and

a lowering of the soil temperature to 18°C . at 8 a.m. On October 18 many of the plants were dead and all were heavily infected with the disease.

Expt. VI, September 15, 1926. In the previous experiments cooling of the soil had been produced by the evaporation of the porous walls of the earthenware pots; in order to make the experiments more conclusive, therefore, an attempt was made to cool the soil in boxes by the application of cold water.

Unfortunately, no means existed at this time of providing heat for the control series, and with the change in wind noted above it was found impossible to maintain the untreated series, i.e. the control, at a temperature above that of the untreated series.

Six boxes, containing nearly a cubic metre of the same soil as used in Expts. II–V, were sown with Ashmouni seed. A preliminary watering was given, sufficient to render the soil moist, after which the following treatments were afforded:

Box (a). Crushed ice applied twice to the surface soil one day after sowing, the soil temperature at 2 in. being thereby reduced to 19.8°C ., but afterwards rising and maintaining an average soil temperature at 8 a.m. of 25°C ., and at 2 p.m. of 28°C .

Box (b). At 10 a.m., three days after sowing, the soil temperature was reduced from 27°C . to 25°C . by iced water, and this temperature was maintained by the application of cold water for four hours, after which no further treatment was given. Subsequent soil temperatures were as Box (a).

Box (c). Similar treatment to Box (b) was afforded five days after sowing.

Box (d). Crushed ice was added as Box (a) seven days after sowing, whereby the soil temperature was maintained at 18°C . for four hours.

Boxes (e) and (f) were untreated, and while the soil temperature was 28.5°C . at 8 a.m., when the experiment was started, it fell two days afterwards to 25°C . owing to decreased humidity.

The amount of infection obtained on the seedling plants on the sixteenth day after sowing was:

Box (a)	64.2 per cent.
(b)	38.8 " "
(c)	26.5 " "
(d)	38.3 " "
Boxes (e) and (f) (controls)	32.5 per cent.

The experiments have been continued weekly throughout the winter, when lower soil temperatures were obtained. During January and part of February the temperatures in the pots ranged from 11.6°C . to 21.6°C ., with a consequent slowing of the rate of development both of the seedlings and of the parasite; three typical observations will suffice.

1st Winter Series.

Sowing date, Dec. 9, 1926.

1st Examination on Dec. 19-20. Soil temps., 14.6° - 23° C.

Infection nil on Dec. 20.

2nd Examination, Dec. 21-31. Soil temps. 15.6° - 23° C.

Infection 9.1 per cent. on Dec. 31.

3rd Examination, Jan. 1-9. Soil temps. 13.7° - 21.6° C.

Infection 26.2 per cent. on Jan. 9.

4th Examination, Jan. 9-22. Soil temps. 17.5° - 24.4° C.

Infection 35 per cent. on Jan. 22.

2nd Winter Series.

Sowing date, Feb. 3, 1927.

1st Examination, Feb. 14. Soil temps. 13.8° - 22.3° C.

Infection nil.

2nd Examination, a week later. Soil temps. from Feb. 14 to 21 were 11.6° - 19.3° C.

Infection 3 per cent.

3rd Examination, a month after sowing. Soil temps. 13° C.- 20.8° C. from Feb. 21 to March 2.

Infection 14 per cent.

3rd Winter Series.

Sowing as shown below :

(a) Ashmouni seed sown on February 8, 1927, in the field and watered normally by irrigation. Soil temps. at 8 a.m. 20.9° C., and at 2 p.m. 31.0° C.

Infection on March 20, 19.8 per cent.

(b) Ashmouni seed sown on February 27 in boxes, the soil of which was maintained by an electric lamp and a thermostat at 28° C.

Infection on March 18, 2 per cent.

Before considering data obtained from the field, three observations of great interest must be considered.

(a) Heavily diseased seedlings from Expt. V (b) were removed to the glass cupboard and the soil temperature raised to 28° - 30° C. Within a few days fresh growth appeared which was to all appearance healthy and remained so for a month.

(b) In September, when the development of the disease was rapid, a series of seedlings seven days old, in which one cotyledon only appeared infected, were deprived of this seat of infection by cutting off the whole cotyledon. These seedlings developed healthily for a month, after which they were all removed to make room for other experiments.

(c) In December lightly infected seedlings were removed from the shed

immediately the lesions were noticed, and placed in the glass cupboard, the temperature of which was raised to 28° C.—30° C., the lesions dried, and the resulting plants showed no signs of disease in January.

FIELD DATA.

As indicated above, the experiments recorded represent a portion only of the work, but are typical of the results obtained.

It will be convenient now to study the data obtained from the field, returning to the laboratory later. Considering first of all the Gezira region, in which the disease has wrought most damage, the past season has been particularly illuminating.

It was realized in August that the prime cause of any serious reduction in the soil temperature during the sowing period was rain, and not irrigation, since observations in the past have shown that after irrigation the temperature of the soil in the upper layers ranges from 28° C. to 30° C. at this time.

Accordingly a visit was paid to two districts of the Gezira Irrigation Scheme on September 6, when the agreement between the incidence of rainfall and the degree of infection was very striking. It was possible to arrive at the approximate date of sowing by inspection of the plots, if the rainfall statistics for the particular district had been first ascertained.

The following details are typical:

Darwish Area.

Rainfall during sowing period, 1926.

July 26	16 mm.	Aug. 5	14 mm.
28	37 "	13	40 "
31	82 "	26	3 "
Aug. 2	5 "	Sept. 2	2 "

Cotton sown on July 27 was heavily infected with bacterial disease and needed resowing. That sown on August 9 was lightly infected, whilst that sown on August 13 was heavily infected.

Gezira Research Farm Area.

Rainfall during sowing period.

July 20	2.5 mm.	Aug. 5	3.0 mm.
25	6.5 "	6	7.0 "
26	12.2 "	13	32.5 "
28	19.8 "	26	a few drops
30	2.9 "	Sept. 2	13.2 mm.
Aug. 1	58.2 "	4	0.4 "
2	4.5 "		

In this area the results are complicated by the fact that the seed was treated before sowing with concentrated sulphuric acid, a procedure which, although not a cure, as indicated above, certainly acts as a deterrent.

Sowing was effected about August 19, and generally speaking the whole area of the farm was remarkably free from the disease; it was found, however, that both self-sown cotton and cotton growing on the water-soaked edges of the irrigation canals were infected.

A little information was obtained from the Gezira Research Farm on the cooling effect of the rains and is included here.

- (1) July 13. Air temperature fell from 38.0° C. to 30.0° C.
Soil " " " 46.0° C. to 25.0° C.
- (2) July 20. Air temperature fell from 35.7° C. to 30.0° C.
Soil " " " 43.0° C. to 25.0° C.
- (3) July 26. Soil temperature fell from 46.0° C. to 24.5° C.

The above were taken in unirrigated soil at 2 in. depth.

The records obtained from another cotton-growing area, e. g. Tokar, are even more informative. This district lies some sixteen miles from the Red Sea coast, and is about sixty miles south-east of Suakin. Sowing takes place in September, following the subsidence of flood-water from the Khor Baraka, and bacterial disease of cotton is almost unknown, although a little must occur from time to time in certain areas of the delta.

The writer was working in this area during the winter of 1916-17, and saw no signs of infection with *P. malvacearum*, and again in 1924 nothing of the disease was seen. Major Archibald paid a visit in 1925 and found a single plant infected with *P. malvacearum*. No careful examination has, however, been made of the crop in the seedling stage, when the disease is easily recognized. It must be mentioned that it is possible for the bacillus to exist in an apparently healthy plant.

Unfortunately soil temperatures were not taken until November, but during this month the soil temperature varied between 28° C. and 32° C., and did not fall until December 17 to 27.5° C., when the winter rains began. By February the soil temperature had fallen to 25.5° C.; an experiment was therefore begun by sowing seed from the same batch as that used in the experiments recorded above, watering the plot by hand.

The experiment was carried out by Mr. P. A. Thompson, Inspector of Agriculture, who kindly furnished the following records:

Sowing date, February 21, 1927. Temperatures taken at 8 a.m.

Temperature of the soil before sowing	25.5° C.
" " " " after "	22.0° C.
" " " " on Feb. 23	25.0° C.
" " " " " " 24	20.0° C.

Temperature of the soil on Feb. 25 22.0° C.

" " " " " 26 23.0° C.

Second watering given.

Temperature of the soil on Feb. 27 22.0° C.

" " " " " 28 22.0° C.

" " " " " March 1 22.0° C.

Third watering given March 3.

Temperature of the soil on March 9 26.0° C.

after which seedlings were forwarded by Mr. Thompson to the writer for examination, and were found to be typically, and heavily, infected with *P. malvacearum*.

Corroborative evidence may be adduced from a study of the cotton crop in other regions (5), but precise data are lacking; wherever the disease is known to be severe, however, the recorded facts fit in with the results described above.

RANGE OF TEMPERATURE FOR GROWTH OF THE BACILLUS ON CULTURE MEDIA.

Returning once more to the laboratory records, a preliminary study was made of the range of temperature in which *P. malvacearum* could grow on culture media. Using a strain isolated from Ashmouni seed it was found that growth almost ceased above 32° C. and did not take place at 37° C. on potato agar.

Satisfactory growth was obtained on potato agar at 11.5° C., the lowest temperature employed, but the most vigorous growth occurred from 20° C. to 24° C.

These results were confirmed with strains isolated from Gezira and Egyptian seed by the writer, and also with a strain isolated by Major Archibald from an infected plant found by him at Tokar.

REACTION OF SAP IN RELATION TO DISEASE.

Bearing in mind the interesting results obtained by Dickson (3) and L. R. Jones (7) on the effects exerted by varying temperatures on the metabolism of the plant, an attempt was made to correlate sap-changes with degrees of resistance by means of the measurement of the hydrogen-ion concentration.

This was effected by crushing the first node of fourteen-day-old seedlings in an agate mortar and adding a single drop of water.

The pH of the liquid was then measured by means of the B. D. H. 'Capillator'. It was recognized that objection might be taken to this procedure, but since a large number of estimations gave similar results within very narrow limits, the method was continued at intervals throughout the season.

These two experiments may lend indirect support to Faulwetter's (4) work, in which he obtained evidence of transmission of the disease in the field from plant to plant by the splashing of raindrops. We have no temperature for actual rainfall in the Gezira, but the apparent coldness of the showers is known to all who have visited that region during the rainy season.

It is hoped to deal with these last aspects of the disease in future papers.

SUMMARY.

The results of the above experiments may be summarized as follows :

1. Evidence has been obtained to show that the development of the bacterial disease of cotton caused by *P. malvacearum* in the seedling stage is confined to a definite range of soil temperature.

2. At soil temperatures of 11–15° C. infection is slow, and in the case of lightly infected seed may be missed.

3. From 16° C. to 20° C. infection is generally obtained, but again it is not usually serious if other environmental conditions are favourable for the growth of the seedling.

4. From 21° C. to 26° C. infection is severe.

5. Above 26° C. to 28° C. the infection again fades in intensity.

6. Above 28° C. little or no infection is obtained.

7. At 30° C. the plant is generally immune.

Experimental evidence suggests that the regional distribution of the disease caused by *P. malvacearum* may be explained on these grounds.

8. Some account has been given of the internal changes associated with varying temperatures, and indirect support is given to Faulwetter's work on the spread of the disease in the field by rain.

The writer is indebted to Mr. M. A. Bailey for suggestions in Experiment VI, to Mr. A. P. Thompson for the sowing experiment at Tokar, and to Mr. N. Macdonald for the preliminary temperature observations with culture media. He also wishes to thank Major R. G. Archibald, D.S.O., for continued interest and helpful criticism during the work.

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On the Carbon Nutrition of some Algae isolated from Soil.

BY

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ALTHOUGH an extensive literature is gradually being built up on the nutrition of many of the lower algae, yet the extent of our knowledge of the physiology of algal species actually isolated from the soil is still very meagre. In 1893 Beijerinck (2) isolated *Chlorella vulgaris* from garden soil, and found it to be identical in physiological properties with the same species isolated from a number of other habitats. He showed that this species was enormously benefited by the presence of organic compounds in the culture media, and that it could be completely independent of light, for it produced a mass of deep green algal material on malt-wort gelatine, even when the culture was kept in the dark for more than a year (3). Since that time a number of other workers (Radais (13), Grintzesco (10), Artari (1), Kufferath (11), Warburg (14)) have isolated the same species from a variety of habitats and studied its physiology from different points of view, and have confirmed and amplified Beijerinck's observations, especially in regard to its independence of light given a suitable organic medium for growth. Chodat (9) has pointed out, however, that certain of the forms identified with Beijerinck's species are probably different strains of the species, since the identification of the organism in most cases has depended solely on its morphological characters, without any attempts at accurate comparison by means of cultures under exactly parallel conditions to those used by Beijerinck; Chodat has found, in fact, that three strains of this species obtained by him from separate localities differ from one another in their finer physiological reactions sufficiently to justify their being regarded as independent varieties of the species.

Charpentier (6, 7, 8) carried out an extensive investigation of the physiology of a unicellular alga which he isolated from soil, and which he identified as *Cystococcus humicola*, Naeg. He found the alga to be inde-

pendent of light in relation to carbon nutrition and to its use of certain nitrogen compounds; but he showed that growth in the dark was always very much less than that in the light under comparable conditions. There is some doubt as to the identity of the alga in question, for Charpentier's description does not sufficiently clearly indicate the nature of the chloroplast, nor mention whether pyrenoids were present or absent, but the fact remains that this organism, like many of the other lower algae, can grow in complete darkness, provided that suitable conditions are available.

Recently Muenscher (12) has experimented, both in diffuse light and in total darkness, with a large species of *Chlorella* originally isolated from soil, and has obtained strong evidence that this alga can synthesize proteins in total darkness when nitrogen is supplied in inorganic combination.

Apart from these species the investigation of the carbon nutrition of the lower semisaprophytic algae has been mainly directed towards organisms isolated from other habitats, such as water polluted with organic matter or lichens; and though these observations are interesting in a general way, there is little reason to believe, in the present limited state of our knowledge, that the species or varieties obtained from soil will be identical in their physiological reactions with those isolated from different habitats.

An account of the relation of certain soil algae to some soluble carbon compounds has already been given by the writer in an earlier paper (Bristol Roach (5)), where it was shown that the addition of small quantities (1 per cent.) of certain sugars, &c., to a mineral salts medium greatly enhances the growth of pure cultures of these species when they are grown in daylight. Further, it was shown quantitatively that one species, *Scenedesmus costulatus*, Chod., var. *chlorelloides*, Bristol Roach, was able to develop and grow in complete darkness if glucose were present in the medium. This type of quantitative work has been followed up during the past two years with especial reference to the influence of light and of glucose on the rate of growth of *S. costulatus*, and it is hoped shortly to embody the results of this work in a separate paper. But a number of more general observations of a qualitative nature have also been made on five different species, and these are briefly summarized below. The experiments were designed to extend to a larger number of species and a wider range of organic compounds the preliminary observations made in connexion with *S. costulatus* var. The experiments up to the present are very incomplete, both in regard to the organic compounds tested and to the basal solution of mineral salts used, but in so far as they give positive results they are of value in throwing more light on the nutrition of the soil algae.

The five species investigated were selected because of their apparently different reactions in the light towards the various sugars tested, and had been obtained in pure culture from a soil sample collected at Geneva, Switzerland. After having been grown for some weeks under purely photo-

synthetic conditions, i. e. on a mineral salts agar medium in the light, each was subcultured on to a number of other agar media consisting of the same basal solution of mineral salts enriched respectively with 1 per cent. of galactose, glucose, mannose, fructose, sucrose, and maltose. Control cultures of each species on the pure mineral salts medium were grown for comparison, and four cultures on each medium were inoculated with equal quantities of a well-shaken aqueous suspension of each species, the algae in the inoculum being so few in number as to be invisible to the naked eye. Two cultures of each species on each medium were left on a table in a room lighted by a north window, and the other two placed in a perfectly dark room. Growth observations on the two series of cultures are summarized below.

PURE CULTURE 16. *Cystococcus* sp.

This species is outstanding on account of the very luxuriant growth that ensues in the light when the medium is enriched with the hexoses, mannite, and most of the disaccharoses, whereas with mineral salts alone growth is much less. The cultures in the light at the end of seven weeks were placed in order of luxuriance,¹ and divided into groups according to the amount of growth, as follows :

luxuriant, (1) glucose, (2) fructose, (3) mannose ;

very good, (4) galactose, (5) sucrose ;

good, (6) maltose ;

fairly good, (7) mineral salts alone.

The cultures in the dark containing sugars were found to compare favourably with those in the light, though they were perhaps not quite so well grown, and were a little lighter in colour. They were classified as follows, and it is seen that the order of availability of the compounds tested is not identical in the dark and in the light :

luxuriant, (1) mannose, (2) fructose and sucrose (equal) ;

very good, (4) maltose ;

fairly good, (5) glucose (cultures obviously staled at an early date) ;

fair, (6) galactose ;

no growth, (7) mineral salts alone.

It seems therefore safe to assume that this species of alga would grow more vigorously under conditions of purely saprophytic nutrition than under those of purely photosynthetic nutrition, and it may be regarded as a true soil alga. In the conditions so far imposed on the organism, no culture in

¹ Cultures in these experiments were classified according to the amount of visible growth into the following groups: (1) luxuriant, (2) very good, (3) good, (4) fairly good, (5) fair, (6) slight, (7) no growth. Some idea of the standards implied by these terms may be obtained from the fact that in a comparison of the growth of twelve species on the basal mineral salts medium in daylight, six were classified as fair, three as fairly good, and three as good.

the light on plain mineral salts agar has ever grown as rapidly as those in the dark with mannose, fructose, sucrose, or maltose, but this does not preclude the possibility that with a more plentiful supply of CO_2 or a greater intensity of illumination, the alga might grow equally well under purely photosynthetic conditions.

At the end of seven weeks' growth in the dark the cultures were placed on the sill of a north window and left for about a month. At the end of that time there was fairly good growth on the pure mineral salts agar, showing that a proportion at least of the inoculated cells had survived their seven weeks' sojourn in the dark. The cultures on the sugary media had all grown considerably, retaining the same order of luxuriance as when grown in the dark, but in every case the colour of the algal stratum was a much more intense green than when grown in the dark.

PURE CULTURE 15. *Chlorella* sp. (different from No. 5).

This species was selected because, while growing uniformly less well in the light than No. 16 on all media tested, the addition of the hexoses and disaccharoses greatly increased the growth of the organism, though on all sugary media there was a tendency to form orange resting cells after a few weeks. After seven weeks' growth in the light the cultures were classified as follows:

very good, (1) glucose, (2) fructose, (3) mannose, (4) maltose;

good, (5) galactose, (6) sucrose;

fair, (7) mineral salts alone.

In comparison with these cultures, the ones grown in the dark showed relatively poor development. The strata were always limited in extent, and the colour varied on the different media from cress green through yellowish olive to Isabella colour, according to an increasing proportion of orange resting cells to green vegetative cells. The tendency already observed in the light to form orange resting cells in sugary cultures was thus found to be considerably enhanced by placing the cultures in complete darkness. Owing to the limited extent of the experiments so far carried out, it is impossible to say to what degree this tendency may be attributable to some possible unsuitability in the basal mineral salts medium, for the source of nitrogen has been observed to have a considerable effect on the growth of certain species in culture (Bristol and Page (4)); more information is therefore required before definite statements can be made in regard to this species. But the fact that there was a certain amount of growth in the dark on all of the media containing organic compounds indicates that this species is quite capable of vegetative growth, for a limited period at least, in the lower layers of the soil. The cultures in the dark were classified as follows:

fair, (1) glucose (Isabella colour¹), (2) sucrose and galactose equal (light yellowish olive¹);

slight, (4) mannose (Isabella colour), (5) fructose (écru olive¹), and maltose (small separate colonies, still cress¹ green);

no growth, (7) mineral salts alone.

A fortnight previously the cultures had been all much greener, and those on glucose, saccharose, and galactose had been comparable in development to those on mineral salts alone in the light. The seven weeks old cultures were transferred from the dark to the sill of a north window, and re-examined a month later, by which time all the cultures had resumed a greener tint and were fairly well grown. They were classified as follows, and it was thought that the change in order of the sugars was probably attributable to the presence in the now better grown cultures of a higher proportion of active vegetative cells when they were replaced in the light:

good, (1) maltose, (2) galactose;

fairly good, (3) glucose [growth arrested or just beginning to be resumed, centre of colony chamois colour shading through olive ochre to warbler green on the margins; a parallel culture left for the whole time in the dark was completely in the resting state (warm buff), and had not made further growth during the last month];

(4) sucrose, mannose, and fructose equal;

fair, (7) mineral salts alone.

The resumption of growth on the sugary media when the cultures were replaced in the light suggests that the increased tendency to enter the resting condition in the dark cannot be wholly due to the effect of the unsuitability of the mineral salts medium, but that this species can probably carry on vegetative growth in the dark only for a limited period of time. Much more work is needed, however, to test the reliability of this suggestion.

PURE CULTURE II. *Scenedesmus costulatus*, Chod., var. *chlorelloides*.

A paper on the growth of this species in the presence of various organic compounds has already been published (Bristol Roach (5)), but some further observations have been made. Seven weeks old cultures in the dark were classified as below:

good, (1) glucose (good at the beginning, but the surface stratum had assumed an orange colour due to presence of resting cells, and a thick streak of cells between agar and glass was beginning to change colour), (2) mannose, but orange cells were evidently beginning to appear;

¹ According to Ridgway's Colour Standards and Nomenclature, 1912.

fairly good, (3) maltose and sucrose (still cress green) and galactose (beginning to become yellow);

slight, (6) fructose (only one écru olive streak near edge of surface);

no growth, (7) mineral salts alone.

Half of the above cultures were left in the dark and the rest placed on the sill of a north window. When re-examined at the end of a month the cultures with glucose, mannose, maltose, and sucrose in the dark had increased very considerably, and were all much greener than at the end of seven weeks, whence it appears that in these cultures the formation of the resting cells was only due to one of the cyclic changes already recorded for this species in cultures grown in the light (Bristol and Page (4)). The parallel cultures to these, placed in the light, showed little or no increase over those left in the dark, and were of the same general appearance though a little darker in colour, with the exception of that containing maltose, in which the organism had developed so luxuriantly in the light as to have produced a deep green stratum equal in extent to that on glucose. The galactose culture in the dark had increased only very little during the last month and the alga had obviously entered into a resting state, whereas the culture in the light was still equal to that on sucrose and was jade to dark cress green. The fructose culture in the dark had obviously made no further growth, and the stratum had become chamois colour; in the light, on the contrary, the algal stratum was quite thick over a limited area of the surface, and of a very deep green colour, far in advance of that with mineral salts alone, where only slight growth was observed. The above observations justify the conclusion that this species is to be regarded as a true soil alga, for it can grow saprophytically in complete darkness on quite a wide range of organic substances, though galactose and fructose do not seem to be equally assimilable in the light and in the dark.

PURE CULTURE 5. *Chlorella* sp. (different from No. 15).

This species was chosen for investigation because it showed, more than any other, conspicuous differences in its reaction towards the different compounds tested in the light. For instance, seven weeks old cultures in the light were luxuriant on maltose, galactose, and fructose, and very good on glucose, starch, and sucrose, whereas growth was only fair on mineral salts alone and lactose, very slight on mannite and glycerine, and completely absent from xylose and mannose, the presumption being that the last four compounds are definitely harmful to the organism.

Seven weeks old cultures on glucose in the dark were found to have grown very well, but there was no growth on any of the other media. Unfortunately some of these cultures became contaminated with fungi, and the results are therefore less reliable than for the other species. The cultures

were transferred to the light, and observed again at the end of a month. The cultures on glucose were then still very good, and one of the cultures on maltose and one with mineral salts had begun to grow. The rest were still blank, though those with sucrose and mannose and one with fructose were badly contaminated.

It seems, therefore, that this species is capable of good growth in the dark, but only under very definite conditions of nutrition, and in the absence of further reliable information it is impossible to define the limits of its activity as a soil alga.

PURE CULTURE 3. *Chlorococcum* sp. (not *humicola*).

This species was selected for trial because in the light it appeared to be almost, or entirely, indifferent to the presence of organic compounds in the medium. Seven weeks old cultures had all produced a thin continuous stratum over the surface of the medium, and when placed in order of luxuriance were found to be arranged in the following order: *good*, (1) galactose, (2) fructose, (3) lactose, (4) glycerine; *fairly good*, (5) maltose, (6) xylose, (7) mannose, (8) mannite, (9) mineral salts alone, (10) glucose, (11) saccharose; but though there was observed to be quite a distinct difference between galactose, the best, and saccharose, the least good, the intermediate cultures changed so gradually from one to the next that it was extremely difficult to detect differences with certainty, and it is possible that such slight differences as appeared may have been accidental, and not due to the sugar present. The slightly depressing action of glucose in the light, however, seems to be a constant phenomenon which has been observed on a good many occasions.

It was interesting to find, therefore, that seven weeks old cultures in the dark had made slight but very definite growth on all of the media tested excepting that with mineral salts alone, so that the depressing action of glucose is not apparent in the dark. In no case was a continuous stratum produced, but pin-head colonies appeared which were cress green or rainette green in colour. The cultures were classified as *fair*, (1) galactose, (2) sucrose; or *slight*, (3) glucose, (4) mannose, (5) fructose, (6) maltose; *no growth*, (7) mineral salts alone; the differences were chiefly in the number of pin-head colonies. When re-examined after being replaced in the light for a month most of these cultures were observed to have made considerable growth; they were classified as follows: *fairly good*, (1) glucose, (2) galactose; *fair*, (3) sucrose, (4) maltose; *slight*, (5) mannose, (6) fructose; *no growth*, mineral salts alone. This is the only occasion on which this alga has been observed to grow better in the presence of glucose than without it, and one is tempted to suggest that the organism had become accustomed to using the glucose in the dark, and that the compound may therefore have

ceased to have a depressing effect when the cultures were replaced in the light. The inability of the alga to resume growth on the mineral salts medium when replaced in the light after seven weeks' sojourn in the dark is interesting, and is possibly due to the fact that at the time of inoculation of the cultures the organism was chiefly present in the zoogonidial stage and contained little or no reserve food material. In the absence of organic compounds in complete darkness the organism in this state would probably die of starvation before it could enter into a resting condition.

The observations made on this alga suggest that it is capable of only limited growth in the dark, but that, provided suitable organic substances are present, it can multiply slowly under saprophytic conditions, and is not merely quiescent. It is possible that a wider series of experiments would reveal the existence of other conditions much more conducive to the luxuriant growth of this organism.

General conclusions. The above cultural observations have been described in detail in order to emphasize a very important fact that has gradually been impressed upon the writer during the last five years, viz. that though the majority of the soil species so far isolated are capable of growing in the dark, provided that suitable organic food is available, yet the requirements of the individual species and their responses to different conditions are extremely varied. It is therefore quite wrong to regard the soil algae as a homogeneous physiological unit, or to argue from one species to another in discussing the relation which these organisms bear to the problems of soil fertility. Still less is it justifiable to apply to the soil algae, as has been done on a number of occasions in the past, facts which have been ascertained, or theories which have been built up in connexion with organisms isolated in pure culture from quite other habitats, until it has been definitely proved that the organisms in the two cases are identical. As an illustration of the dangers involved in making general statements from superficial resemblances of organisms to one another, the following observations may be cited: from the same soil sample as yielded the original pure culture of *Scenedesmus costulatus*, Chod., var. *chlorelloides* (No. 11), a second pure culture was obtained (No. 6) of an organism which for two years was regarded by the writer as a duplicate culture of the same organism. Microscopically it appeared to be identical, and when subcultured on the usual range of sugary media its reactions and the form and colour of the colonies differed so little from those of the type culture that the two forms were assumed to be identical. When, however, the two forms were grown as shake cultures in gelatin media a difference in physiological reaction soon became apparent, for whereas No. 11 slowly but completely liquefied the medium as it developed, forming eventually a continuous green stratum which gradually sank within the medium as the underlying gelatin became attacked and liquefied, No. 6 grew in isolated pin-head colonies, and

even at the end of twelve months there was no sign of liquefaction of the gelatin. Obviously the relation of these two strains to soil fertility is likely to be very different, and the writer would strongly emphasize the necessity for accumulating physiological data of this kind before any attempt is made to generalize on so complex a subject.

SUMMARY.

An account is given of the growth, both in daylight and in complete darkness, of five species of soil algae, isolated in pure culture, on media containing mineral salts enriched with various sugars. It is shown that all five species are capable of growing in complete darkness, provided that a suitable organic compound is present in the medium, and may therefore be regarded as true soil algae, but that the five species react quite differently to the conditions imposed upon them, and that they vary considerably in the extent to which they are able to grow in the dark. It is concluded that it is not justifiable to regard the soil algae as a homogeneous physiological unit in considering the relation which these organisms bear to the problems of soil fertility.

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Studies in the Genus *Fusarium*.

V. Factors determining Septation and other Features in the Section *Discolor*.

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With twenty-seven Figures in the Text.

THE recent work of Brown and Horne (2) on the genus *Fusarium* has shown that both the degree of septation of the spores and the nature of their content can be modified either by altering the concentration of the nutrient medium used, or by altering the ratio of certain constituents of the medium. Thus it was found that low septation is produced by high concentration of the nitrogen-containing constituent of the nutrient medium, by the presence of an unduly high concentration of acid or alkali and other factors. The ratio of the concentrations of the carbon and nitrogen-containing constituents is also of considerable importance in determining septation and other sporal characteristics. These authors confined themselves, however, to a group of strains belonging to only one species of *Fusarium*. The object of the present work was to determine whether the same relation between sporal characteristics and the nutrient medium obtained when the scope of the investigation was widened to include several species of *Fusarium*. Since the grouping of the species of *Fusarium* depends primarily upon sporal characters, it was decided to choose a group of species which appeared to differ sharply in these respects from the strains studied by Brown and Horne. Accordingly, after a preliminary study of several species of *Fusarium* belonging to different sections of the genus the work was limited to certain species belonging to the Section *Discolor*. According to Sherbakoff (5), species included in this Section are characterized by their spores, which are cylindrical, broadened towards the apex, and abruptly attenuated at the apical end. They

produce intercalary but not terminal chlamydospores. They do not form microconidia. From the point of view of spore shape, the Section is stated to occupy an intermediate position between the Sections *Elegans* and *Martiella*. With *Fusaria* of the Section *Elegans* the spores are more or less gradually pointed towards the apex. This feature is also fairly characteristic of the group of strains studied by Brown and Horne.

The cultures used in the investigation were obtained from different sources. Certain cultures which had awaited identification at the Imperial College for some time were submitted to Dr. Wollenweber and referred by him to the Section *Discolor*, others were kindly sent by Dr. Wollenweber for the purpose of this work, and others were obtained from the Centraal-bureau voor Schimmelcultures, Baarn, Holland.

A list of the available strains and the special notation assigned to them is given below:

- CB. *Fusarium culmorum*, (W. G. Smith) Sacc., obtained from Baarn, 1925.
- CC. *Fusarium culmorum*, (W. G. Smith) Sacc., isolated by Mr. K. St. G. Cartwright from the wood of *Fraxinus excelsior* in 1923, at the Imperial College of Science, London. Identified by Dr. Wollenweber in 1925.
- CJ. *Fusarium culmorum*, (W. G. Smith) Sacc., isolated by Mr. Howard Jones from the fruit of *Ribes grossularia* in 1924. Identified by Dr. Wollenweber in 1925.
- D. *Fusarium discolor*, App. et Wr. (= *F. sambucinum*, Fuckel), obtained from Baarn, 1924.
- L. *Fusarium lolii*, (W. G. Smith) Sacc., obtained from Baarn, 1925.
- P, P¹. *Fusarium polymorphum*, Matruchot. Strains obtained from a culture sent by Dr. Wollenweber (1925). The fungus was originally isolated from pionnotes occurring on *Secale cereale*, Dahlem, Germany.
- Pp. *Fusarium polymorphum* var. *pallens*, Wr. Sent by Dr. Wollenweber (1925). Originally isolated from the base of the stems of *Dianthus caryophyllus*, Pillnitz, Saxony.
- Sa. *Fusarium sambucinum*, Fuckel (*F. discolor*, App. et Wr.) Sent by Dr. Wollenweber (1925). Originally isolated from *Rubus idaeus*, Beyerland, Holland.
- SG. *Fusarium sulphureum*, Schlechtendahl (*F. discolor* var. *sulphureum*, (Schlechtendahl, sub specie) App. et Wr.). Sent by Dr. Wollenweber (1925). Originally isolated from the bark of *Robinia pseudacacia*, Potsdam.
- SB. *Fusarium sulphureum*, Schlechtendahl. Culture labelled *F. discolor* var. *sulphureum* obtained from Baarn, 1925.
- T. *Fusarium trichothecioides*, originally obtained from Amsterdam.

At the commencement of the investigation all the above-mentioned strains were subcultured on the medium referred to subsequently as the standard medium, and the new cultures were kept in the dark at 20° C. Cultures of monosporous origin were obtained from these, and the strains were again subcultured on the standard medium, using inocula from the monosporous cultures. As a result of these preliminary experiments it was found that all the strains were different, not excepting the strains of *F. sambucinum* and *F. sulphureum* derived from Germany and Holland respectively. During the course of the work a number of saltants were obtained, chiefly from the parent strains CC, CJ, P, and SG, and it was found that the saltants derived from a given parent showed, in some cases, less resemblance in morphological characters than that apparent between any two of the parent strains. In these circumstances it was considered advisable to make a comparative study of the various parent and saltant strains, but since this aspect of the *Fusarium* problem was outside the scope of this work, the investigation was undertaken by one of the present authors (J. H. Mitter).

The general methods employed in this investigation were those which were found suitable in the previous work (2) for the microscopical study of the spores of *Fusarium*, especially as regards the nature of the spores and their septation. It was not possible, however, to rely on the results obtained from septation counts of fifty spores, owing to the degree of variation in the septation of spores situated at equal distances from the centre of the colony which was found with certain strains. Counts made from at least one hundred, and frequently two hundred or even more, spores were necessary to ensure accuracy.

The result of six successive counts of 100 spores for the strain CC after twenty-four days is given in Table I. In four out of the six counts a well-marked 4-mode is shown, while the average for the whole 600 yielded a 4-mode. With one exception (count showing a 3-mode) the figures obtained for the average septation are in fairly close agreement.

TABLE I.

	1	2	3	4	5	6	Average Septation.
1st 100	0	2	33	50	15	0	3.78
2nd "	1	1	50	36	12	0	3.57
3rd "	0	0	33	60	7	0	3.74
4th "	0	2	30	54	14	0	3.80
5th "	0	5	27	57	11	0	3.74
6th "	1	3	39	36	20	1	3.74
Mean	0.3	1	35.3	48.8	13.1	0.2	3.73

The difficulty of counting the septa of spores of the granular and vacuolate types was overcome as in the previous investigation (1) by the use of ruthenium red.

In order to eliminate the factor occasioned by the variation in septation

in a radial direction, the spore samples were taken almost invariably from a point situated 1 cm. from the centre of the colony.

The records of septation put forward in this investigation are based on counts of about 40,000 spores.

A. GROWTH-RATE AND SEPTATION IN RELATION TO DILUTION OF THE STANDARD MEDIUM.

The standard medium adopted in this investigation was of the same composition as that used in the earlier work (2), except that starch was added. It consisted of the following ingredients:

Glucose	2	grammes
Potato starch	10	„
Asparagin	2	„
K ₃ PO ₄	1.25	„
MgSO ₄ , 7H ₂ O	0.75	„
Agar	15	„
Water	1	litre

The standard medium of the normal strength will be denoted by the letter N. The changes in the growth-rate and degree of septation were studied in relation to the following modifications of N, e. g. 2 N, N, N/2, N/4, and N/10. Septation counts were made after an average interval of twelve days. In order to take into account the time factor, further counts were made after a second average interval of twelve days.

All the Discolor strains examined, with the exception of P and P¹, exhibit the non-staling type of growth, that is to say, the rate of 'radial advance'¹ is practically uniform. This point is shown by the curves given in Fig. 1, which have been obtained by plotting the length of the radius of the colony against time. It will be observed that the graphs for the strains represented in the figure make different angles with the base line, indicating that the rate of radial advance varies for different strains. In the case of all the strains, the lower slope of the curve which is apparent up to the end of the second day is due to the fact that a short time allowance should be made for spore germination; after this interval of time the gradient increases and remains fairly constant.

The average rates in cm. per day for the strains CC, CJ, D, and L (see Fig. 1) in various concentrations of the standard medium are given in

¹ The expression 'radial advance' is used by Gregory and Horne (8), in dealing with the invasion of apples by fungi, to indicate the rate of advance inwards of the invading fungus in a radial direction from the point of infection on the circumference of the fruit. In the present investigation the expression is used for the rate of progress of the fungus in plate cultures, measured from the centre of the colony in a radial direction outwards.

Table II. These figures are obtained by averaging the daily differences in radial advance obtained after the first two days.

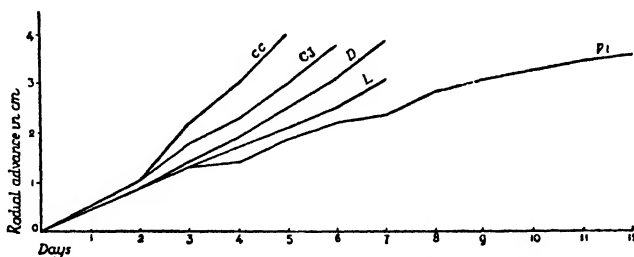
FIG. 1. Graph showing rate of radial advance for strains CC, CJ, D, L, and P¹ (standard medium).

TABLE II.

Strain.	2 N.	N.	N/2.	N/4.	N/10.
CC	1.1	1.3	1.3	1.3	1.0
CJ	0.82	0.78	0.72	0.68	0.72
D	0.69	0.71	0.67	0.68	0.64
L	0.49	0.43	0.44	0.44	0.40

The rate of radial advance in the standard medium is not uniform, however, in the case of the strains P and P¹. Here the gradually decreasing gradient shown by the curve (Fig. 1) indicates that the rate is progressively retarded, a feature consistent with staling in cultures.

Since the majority of the strains under consideration exhibit the unstaled type of growth, it might be anticipated from Brown's results (1) that the rate of radial advance would not be greatly affected by altering the concentration of the medium. The results obtained are in accordance with this expectation. Nevertheless, certain minor variations, due to altered concentration, are apparent with certain strains. The chief types of variation experienced are shown graphically in Fig. 2, where the rate of radial advance is plotted against concentration for the strains CC, SB, and L.

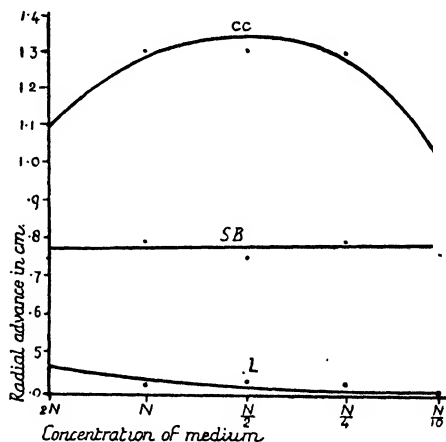


FIG. 2. Illustrating the relation between rate of radial advance and altered concentration of the standard medium.

With strain SB the rate remains fairly constant over the whole range, with strain CC the rate rises to a maximum at strengths N-N/4, and

declines towards N/10, and with L there is a slight decrease in the rate towards N/10.

The influence of altered concentration of the standard medium on the septation shown by the Discolor strains is illustrated graphically in Fig. 3, where the percentage number of spores having a given number of septa (0-8) is shown by the height of the columns raised from the base line.

With the strain CJ (Fig. 3) little change in the septation occurs throughout the whole series. With the strain SG, where the average septation shows a progressive rise from 2.98 (2 N) to 4.37 (N/10), a high 3-mode is present at the concentration 2 N, and a high 5-mode at N/10, due to a gradual decrease in the number of 3-septate spores associated with a corresponding increase in the 5-septate spores. In the case of strain P¹ a 5-mode occurs throughout the series. This mode, however, rises to a maximum at N/4 and falls towards N/10. Correspondingly the average septation rises from 4.20 to 4.64 (N/4) and falls to 4.06 (N/10). The greatest change in the septation is shown by the strain T. At 2 N a double peak is shown owing to the preponderance of 1- and 3-septate spores in almost equal numbers; at N/2 a high 3-mode is present, changing at N/4 and N/10 to a high 5-mode. Correspondingly the average septation changes from 1.99 (2 N) to 4.5 (N/10). The last case considered, that of the strain L, is exceptional. A double peak is shown throughout the series, caused by the great preponderance of 1- and 3-septate spores. It is also clearly evident that the number of 3-septate spores diminishes, and is associated with a corresponding increase in the number of 1-septate and 0-septate spores with progressive dilution of the medium. Correspondingly the average septation *progressively decreases* with increased dilution (2.29-zero). In Fig. 5 the changes in the average septation for the various strains considered above are represented graphically.

The chief time effects in relation to septation are shown in Table III, which gives the result obtained from spore counts made after twelve and twenty-four days' growth respectively.

The septation changes undergone by the various strains in time, recorded in the table, may be summarized as follows:

Strains CJ and CB. Very little change.

Strain P¹. A fall in all except concentration N/10. The general distribution of septation throughout the series remains the same.

Strain SG. The septation rises at all concentrations.

Strain Sa. A marked rise occurs at concentrations N/4 and N/10.

Strain L. A slight rise occurs.

Strain T. A fall in the septation with its maximum effect at concentrations N/4 and N/10, completely altering the distribution of septation throughout the series.

Concentration of medium
2N

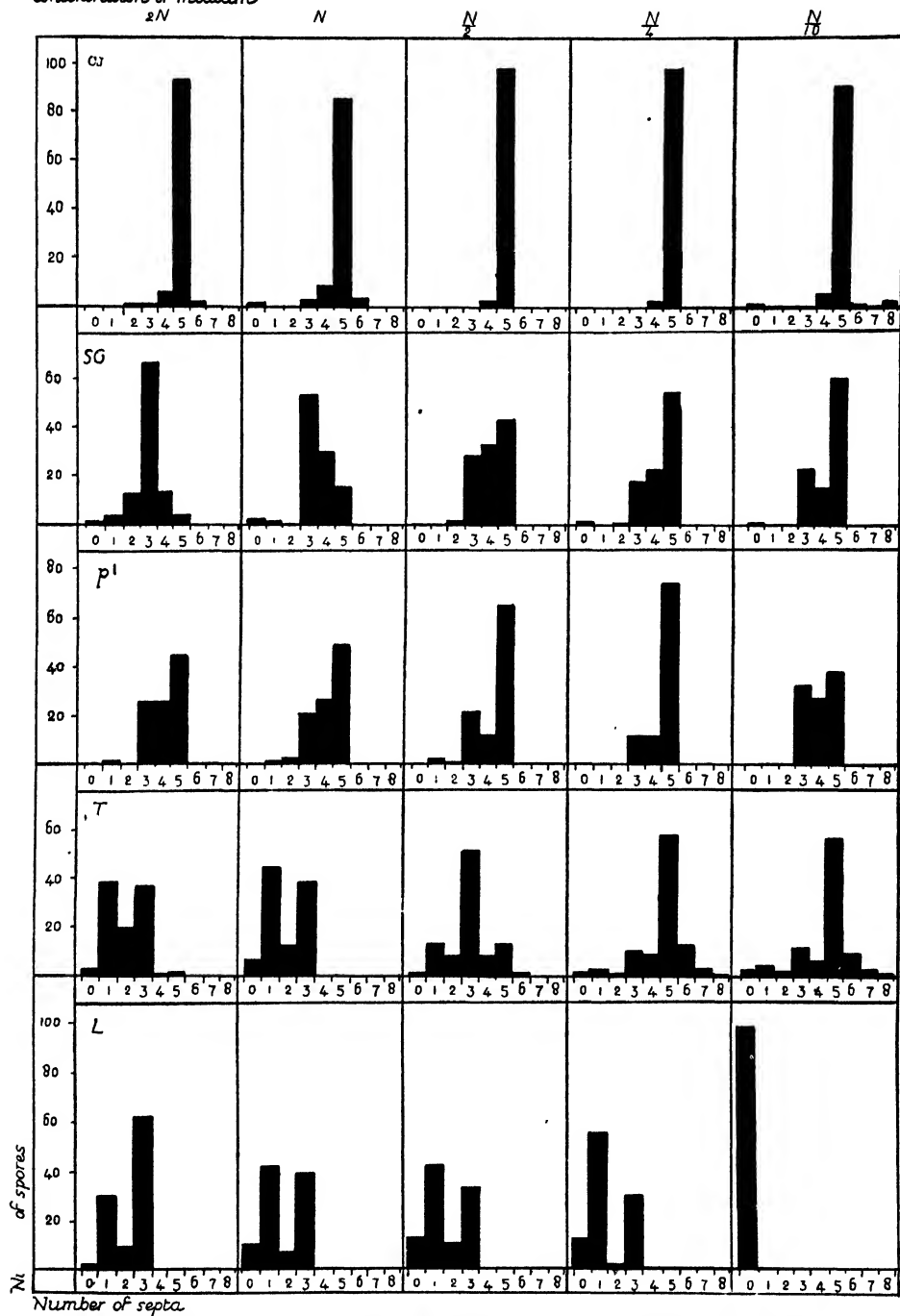


FIG. 3. Series 2N-N/10. Graphical representation of the distribution of septation as shown by the strains CJ, SG, P¹, T, and L.

TABLE III.

Septation in Relation to Time.

Strain.	Conc. of Medium.	12 Days.		24 Days.	
		Mode.	Average Septation.	Mode.	Average Septation.
CJ	2 N	5	4.92	5	4.93
	N	5	4.87	5	5.0
	N/2	5	4.98	5	4.99
	N/4	5	4.98	5	5.01
	N/10	5	4.97	5	5.03
CB	2 N	4	3.85	4	3.8
	N	5	3.99	4	3.95
	N/2	5	4.20	5	4.18
	N/4	5	4.17	5	4.51
	N/10	5	4.64	5	4.74
L	2 N	1, 3	2.29	—	—
	N	1, 3	1.74	1, 3	2.03
	N/2	1, 3	1.61	1, 3	1.98
	N/4	1, 3	1.5	1, 3	1.57
	N/10	0	< 1.5	0	< 1.57
SG	2 N	3	2.98	3	3.66
	N	3	3.53	5	4.31
	N/2	5	4.15	5	4.36
	N/4	5	4.35	5	4.53
	N/10	5	4.37	5	4.61
P ¹	2 N	5	4.20	5	3.86
	N	5	4.25	5	4.17
	N/2	5	4.41	5	4.28
	N/4	5	4.64	5	4.27
	N/10	5	4.06	5	4.06
Sa	2 N	4, 5	4.05	5	4.14
	N	5	4.57	5	4.48
	N/2	5	4.70	5	4.75
	N/4	5	4.51	5	5.0
	N/10	5	4.38	5	4.86
T	2 N	1, 3	1.99	1, 3	1.71
	N	1, 3	1.81	1, 3	1.74
	N/2	3	2.97	1, 3	1.77
	N/4	5	4.7	1, 3	2.47
	N/10	5	4.5	1, 3	2.59

In studying the septation changes undergone in time by the various strains, two points must be borne in mind :

(1) The influence of the medium or particular concentrations of the medium on spore development. With some strains, for example CJ, the spores may obtain their full complement of septa relatively early ; with others (Sa, SG) relatively late.

(2) The influence of the medium as changed through the growth of the fungus on the fully developed spores and on those produced later in the older portions of the culture. This influence is shown in various ways :

- By the development of an increasing proportion of small spores of low septation in spore samples taken from time to time.
- By the occurrence of atrophied spores associated with sporal chlamydospore formation.
- By the occurrence of spores in a state of fragmentation. Two types

have been observed, the first in which the spore segments became rounded, and the spore presented a beaded appearance (usually associated with granularity of the spore content); the second in which constriction appears at the middle septum, and the spore separates at this point into two comma-shaped portions.

The time when these effects become manifest varies with different strains.

In studying septation in relation to altered concentration of N, Brown and Horne found that high septation was correlated with low concentration, and when the concentration was increased the average septation declined, the point at which the decline occurred depending on the strain employed. A certain amount of evidence was obtained which showed that if the concentration was sufficiently low, the septation declined with decreased concentration. The general effect, therefore, might be represented by an optimum curve with gradients descending in the direction of a high and low concentration of the medium. The results obtained in the present investigation support this view. The curve shown for P¹ (Fig. 4) is clearly an optimum curve. That given for SG shows the summit and left-hand descending portion (in the direction of increasing concentration) of such a curve. Those given for CB and T show the left-hand descending portion only. With the strain CJ no change in the septation occurs throughout the whole range ($2N$ – $N/10$). With this strain the effect of altering the concentration of the medium is not always reflected in the septation, the change affecting the capacity for producing spores rather than the degree of septation: see the results described subsequently for the glucose series. The curve obtained for the strain L is strikingly unlike the others. Only the descending right-hand portion of the optimum curve is represented, the septation declining from $2N$ to $N/10$.

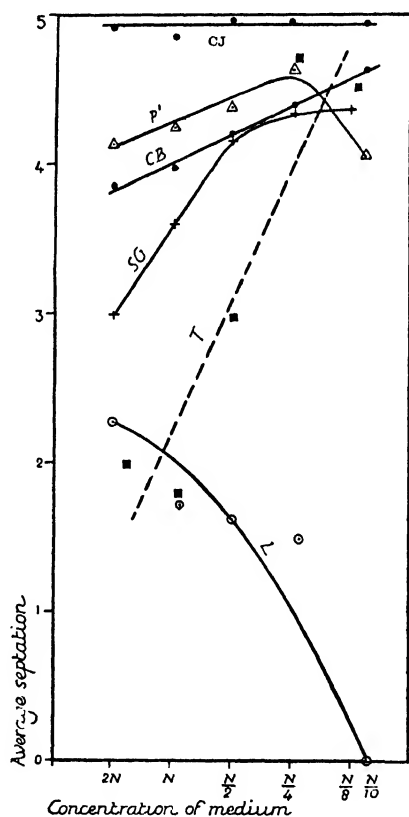


FIG. 4. Series 2 N–N/10—graph showing average septation curves for the strains CJ, CB, SG, P¹, T, and L.

B. GROWTH-RATE AND SEPTATION IN RELATION TO ALTERED CONCENTRATION OF THE CARBON CONSTITUENT.

When the Discolor strains were grown in the standard medium with starch omitted, and the glucose constituent varied from two to eight per cent. with a view to studying the colours produced by the various strains, it was observed that the higher concentration had a marked effect on spore production and septation. Certain strains (CJ, CB, &c.) produced an abundance of spores with relatively high average septation, others produced relatively few spores, and the average septation was low (P, &c.), whilst a few failed to sporulate (Sa). The more important observations made on the behaviour of the strains are summarized below.

(a) No spores observed.

- | | |
|--------|---|
| Strain | L. Neither spores nor chlamydospores observed. |
| ,, | SG. Neither spores nor chlamydospores present. Mycelium highly granular. |
| ,, | D. Neither spores nor chlamydospores present. Mycelium somewhat granular. Only one spore (attached to a basal cell) observed. |
| ,, | Pp. No spores. Terminal and intercalary chlamydospores present. |
| ,, | T. No typical spores observed. Few highly granular and irregular spores. |

(b) Spores few, or when present the average septation is low.

- | | |
|--------|---|
| Strain | P. Spores very scarce, 0-3 septate (1-septate forms predominate). Terminal and intercalary chlamydospores present. Mycelium beaded. |
| ,, | P ¹ . Some spores present (about 30 μ long), 0-3 septate. No chlamydospores observed. Mycelium presents a beaded appearance owing to the presence of highly refractive granules in the hyphae. |
| ,, | Sa. Spores very few, highly granular, 0-5 septate. Long granular spores, constricted at the septa, occur. Terminal and intercalary chlamydospores few. |
| ,, | SB. Spores numerous, 0-3 septate. Average septation 1.10. Two types of spore are present: (1) Strongly constricted highly granular spores, 1-3 septate; maximum 40 $\mu \times 7 \mu$. The apical segment is often bent and ovoid in shape. Round, ovoid, and comma-shaped single cells occur, which appear to have been derived from spores of this type. (2) Shorter spores (6-30 $\mu \times 3 \mu$ approx.), the septation of which is difficult to determine; the majority are 0-septate. Intercalary chlamydospores present. |

(c) Spores numerous, average septation fairly high.

Strain CB. Average septation 3.53, 3-mode.

„ CC. Average septation 3.75, 3 to 4-mode.

„ CJ. Average septation 4.54, high 5-mode.

The above observations were made when the cultures were twenty-one days old.

In order to investigate the effect of altered glucose concentration on spore production and septation, series of experiments were carried out in which the concentration of the glucose constituent (G) of the standard medium was varied from 2 to 20 per cent. (2 G–20 G), using certain strains (CB, CC, CJ) which had exhibited moderate or strong spore development, and others (P, P¹, Pp) which had produced few or no spores at all at 8 G.

TABLE IV.

Strain.	Glucose Per Cent.	0	1	2	3	4	5	6	7	Average Septation.
CB	2	0	1	0	14	28	55	2	0	4.42
	8	1	7	6	40	17	28	1	0	3.53
	10	0	5	6	35	27	27	0	0	3.65
	14	4	6	3	45	17	25	0	0	3.40
	16	0	7	6	41	26	19	1	0	3.47
	18	3	3	4	58	23	9	0	0	3.22
	20	1	2	5	73	13	16	0	0	3.13
CC	2	0	0	0	12	21	66	0	1	4.57
	8	0	0	1	40	42	17	0	0	3.75
	14	1	1	8	40	31	19	0	0	3.56
	16	0	3	5	68	19	5	0	0	3.18
	18	2	10	10	46	17	15	0	0	3.11
	20	1	3	13	65	17	1	0	0	2.97
CJ	2	0	0	0	1	5	92	3	1	4.98
	8	0	0	0	14	18	68	0	0	4.54
	14	1	2	0	11	31	55	0	0	4.34
	16	2	0	2	16	6	74	0	0	4.46
	18	0	0	0	8	14	78	0	0	4.70
	20	0	0	0	8	18	74	0	0	4.66

In Table IV the septation shown by the strains CB, CC, and CJ at glucose concentrations ranging from 2 to 20 per cent. after twenty-five days' growth is given. From the table it is clear that very little change in the average septation has taken place in the case of the strain CJ; with CB and CC, however, the average septation gradually declines with increasing concentration. The effect of an increasing glucose concentration cannot, however, be gauged from a study of the changes in the septation alone. The general effect on spore production must be taken into account.

The relation of sporing capacity to septation is illustrated in Fig. 5, where the average septation for the 2 G–20 G series is plotted for the strains CB and CJ from the data given in Table IV. The dotted lines indicate very approximately the decline of spore production from the full (100 per cent.) capacity which accompanied increased glucose concentration. These lines are based mainly on observation of the relative prevalence

of spores at the various concentrations. With CB, and the same holds good for CC, the reduced sporing capacity is associated with a decline in the average septation, a decline partly due to the occurrence of small spores of low septation at the 8 G and higher concentrations. With CJ, however, the average septation remains unchanged, with diminishing spore production until sporulation ceases (see Table IV).

The strains of the classes (*a*) and (*b*) (sporing feebly or not at all at 8 G) were grown in serial plate cultures where the glucose concentration

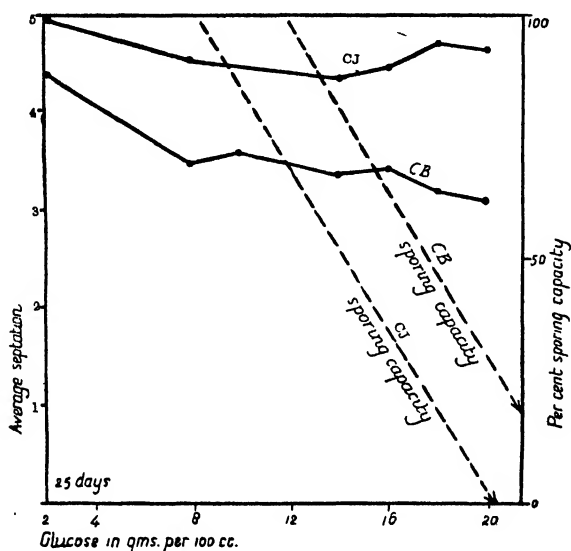


FIG. 5. Graph showing the effect of varying the concentration of the glucose constituent of the standard medium on sporing capacity and septation in the case of strains CJ and CB.

ranged from 2 to 10 per cent. (2 G–10 G). Only the data obtained for the strains of *Fusarium polymorphum* (P, P¹, and Pp) will be given in detail, since they are typical of those yielded by this particular group of experiments (see Fig. 6).

Strain P. There is a high 5-mode and extremely few small spores of low septation at 2 G. The spores were very uniform as to length and shape. At 4 G the 5-septate spores have decreased in number with a corresponding increase in the 3's and 4's. The spores are still fairly uniform. At 6 G a high 3-mode occurs with greatly diminished 4's and 5's. At 8 G a 1-mode occurs. The spores exhibit lack of uniformity and small spores predominate. At 10 G very few spores are present and the septation is approaching zero.

Strain P¹. At 2 G the spores are practically all 5-septate and of uniform type. At 4 G a 3-mode is present and spores of low septation

(1–2 septa) are in excess of the 4's and 5's. These small spores increase numerically until at 8 G a moderately high 0-mode occurs. At 10 G the septation has reached zero.

Strain Pp. At 2 G a moderately high 5-mode is manifest, and no spores of low septation are present. At 4 G a 3-mode is evident and

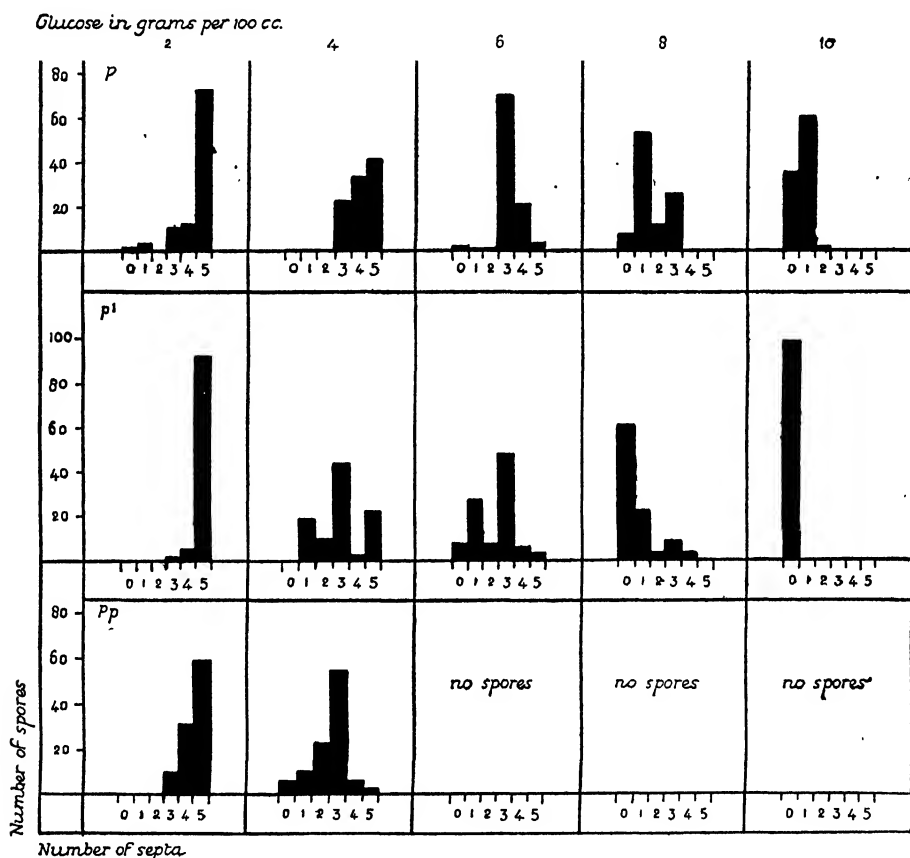


FIG. 6. Glucose series. Graphical representation of the distribution of septation as shown by the strains P, P¹, and Pp.

numerous small spores occur. Spores were absent at higher glucose concentrations.

The different ways in which the three strains in question react towards increased glucose concentration are shown graphically in Fig. 7. With these strains reduction in the sporing capacity almost exactly corresponds with the decline in the average septation.

A comparison of Figs. 5 and 7 will show the striking difference in the behaviour of the strains *Fusarium polymorphum* on the one hand, and the strains CB and CJ (*F. culmorum*) on the other hand.

The effect of the time factor on septation is well shown by the following examples:

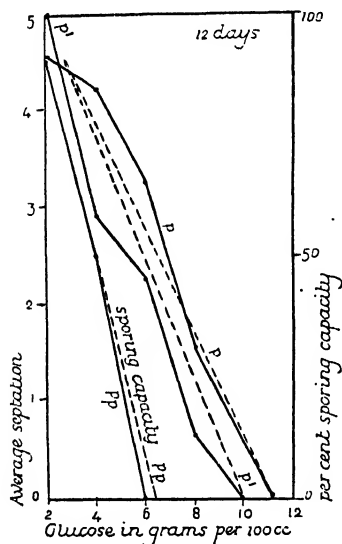


FIG. 7. Graph showing the effect of varying the concentration of the glucose constituent of the standard medium on sporing capacity and septation in the case of strains P, P¹, and P_p.

Strain CC. In Fig. 8 the septation observed at 14 G and 18 G respectively is given for different time intervals, viz. 12, 15, 25, and 35 days. It will be seen from the figure that the column representing 5-septate spores, at 14 G, reaches its maximum height on the 15th day (41 per cent.), is much shorter on the 25th day (19 per cent.), and very short on the 35th day (3 per cent.). From the 15th day onwards the 3-septate column rises and spores of low septation became increasingly evident. At 18 G more spores of low septation (about 20 per cent.) were evident on the 12th day than at the lower concentration. On this date these spores had not attained their full complement of septa. On the 15th day few spores of low septation occurred and spores of higher septation show an increase. On the 25th day and onwards short spores of low septation had increased numerically. The

spores of low septation were due in both cases to the operation of degenerative processes. The behaviour of the strain CC at the two glucose concentrations in question is graphically represented in Fig. 9, where the average septation is plotted against time.

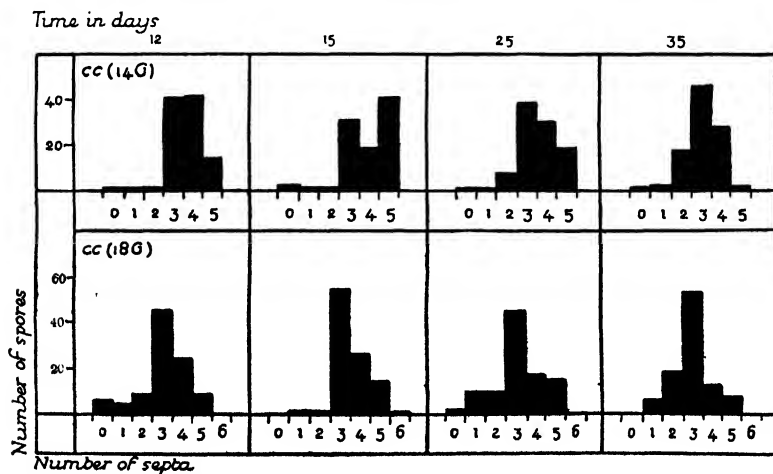


FIG. 8. Graphical representation of the change in the distribution of septation in time as shown by strain CC at different glucose concentrations (14 G and 18 G).

Strain CJ. In Fig. 10 the average septation is plotted against concentration after three time intervals, viz. 15, 25, and 35 days. It will be seen that on the 15th day a fall in the average septation occurs from 14 G to 18 G. On the 25th day the fall is eliminated, by which time the spores have attained their full complement of septa. On the 35th day it was

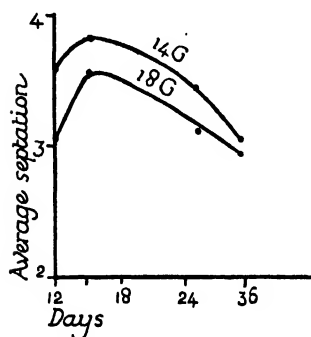


FIG. 9. Graph illustrating the effect of time on the average septation as shown by the strain CC at different glucose concentrations (14 G and 18 G).

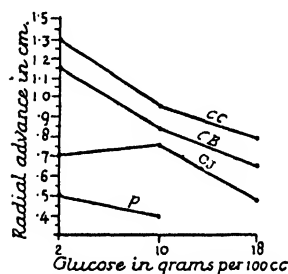


FIG. 11. Graph showing the effect of varying the glucose concentration on the rate of radial advance (strains CC, CB, CJ, P).

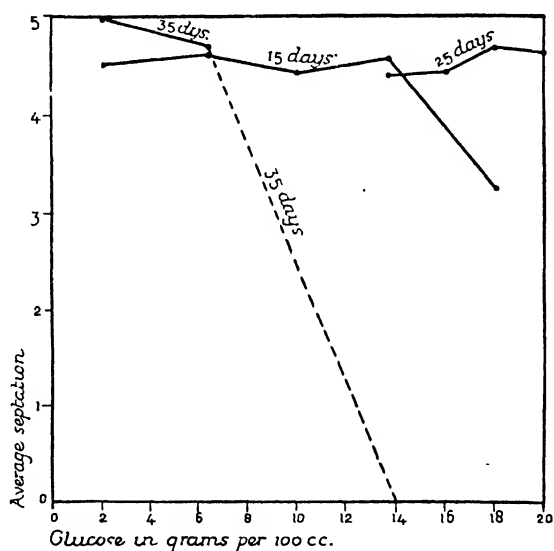


FIG. 10. Glucose series. Graph showing the effect of time on the average septation curve (strain CJ).

impossible to obtain an accurate estimate of the average septation beyond 6 G, owing to the operation of degenerative processes.

With regard to colony growth the average rate of radial advance tends to diminish with increased concentration. This point is represented graphically in Fig. 11 for the strains CC, CB, CJ, and P, where the average rate of radial advance is plotted against concentration.

An effect of increasing the glucose concentration is seen in the development of granularity in the spore contents. The concentration at which granularity appears varies for the different strains. Granularity is usually evident at 6 G; it develops with increasing concentration and tends to obscure the septation. Varying the glucose concentration appeared to have

very little effect on chlamydospore formation, which is of fairly general occurrence among the Discolor strains. Special attention was given to the strain CJ, which produces very numerous, dark, warty chlamydospores; these, however, were found to be very numerous throughout the whole range of concentration employed. It was difficult to determine whether any numerical change was associated with altered concentration.

All the strains dealt with in this paper, with possibly three exceptions, show fairly strong colour development at 8 G. The predominant colour at this concentration is usually some shade near pomegranate, purple, or Bordeaux (Ridgway's colour standards). The minimum distribution and intensity of colour occur at the lowest concentration: they increase up to 8 G or 10 G, but usually decrease at higher concentrations.

C. SPORE DEVELOPMENT AND SEPTATION IN RELATION TO ALTERED CONCENTRATION OF THE NITROGEN CONSTITUENT.

In this work the standard medium was modified by altering the concentration of asparagin. The standard medium contains 2 gm. asparagin per litre. The asparagin content of the series chosen ranged from 0.1 to 16 gm. per litre or from $\frac{1}{20}$ to 8 times the concentration present in the standard medium. As with the experiments described in the preceding sections of this paper, the culturing was carried out under standard temperature conditions (20° C. approx. in the absence of light). The septation records were made after an interval of from 12 to 18 days, and in some cases an additional record was made after a longer time interval. For descriptive purposes the strains will be dealt with in groups of presumably closely related forms.

Group 1. Strains SB, Sa, and D (Figs. 12 and 13).

Strain SB. Range 0.1 A–16 A, viz. asparagin concentration 0.1–16 gm. per litre.

At 2 A, the normal standard medium, a high 5-mode is evident with very few 4's and 5's (see Fig. 12). Passing down the series in the direction of reduced asparagin, a marked change occurs at 0.2 A, where the 5-mode is replaced by a moderately high 3-mode, but about 40 per cent. of the spores are 4- and 5-septate. Finally at 0.1 A the 4's and 5's are reduced in number to about 5 per cent., whilst a number of short spores of low septation are present. Passing up the series in the direction of increased asparagin, the 5's decrease steadily to concentration 4 A. A sharp decline in the septation occurs at 8 A, where the 5-mode is replaced by a 3-mode. At 16 A the 4's and 5's are reduced in number with a corresponding increase in the 3's and spores of low septation. By plotting the average septation against concentration a curve of the usual optimum type is obtained (Fig. 13), in this case with a wide arch between 0.5 A and 2 A, and descending sharply on both sides towards the highest and lowest concentrations respectively.

Asparagin in grams per litre

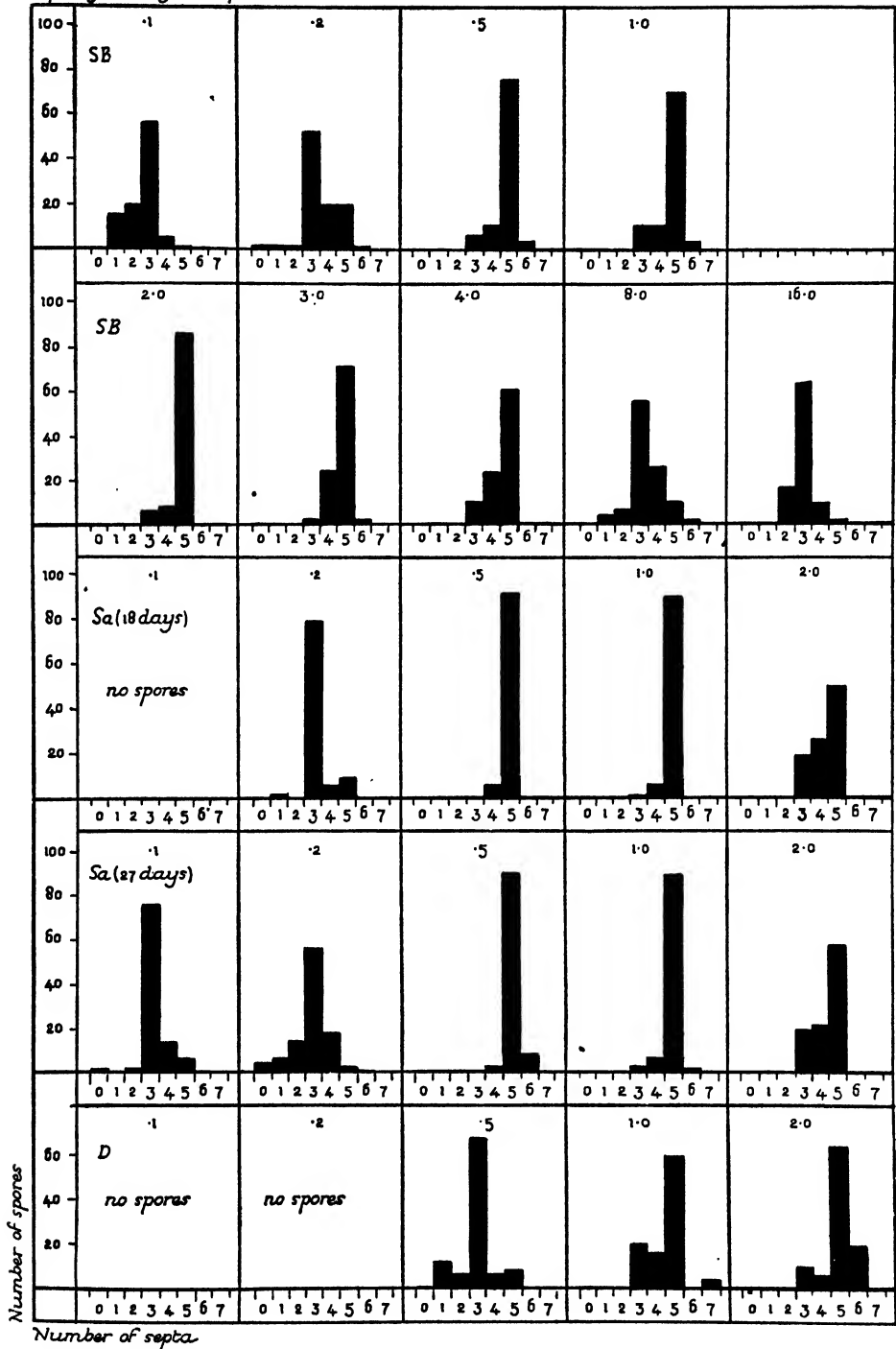


FIG. 12. Asparagin series. Graphical representation of the distribution of septation as shown by the strains SB, Sa, and D.

Strain Sa. Range 0.1 A–2 A.

Fig. 12 shows the modal changes as recorded after 18 and 27 days respectively. A high 5-mode occurs at 0.5 A and 1 A; with decreasing asparagin the 5-mode is replaced by a 3-mode at 0.2 A, while at 0.1 A spores were absent (18th day). The curve for the average septation shown in Fig. 13 (27 days) is clearly of the type shown for SB, but the arch is

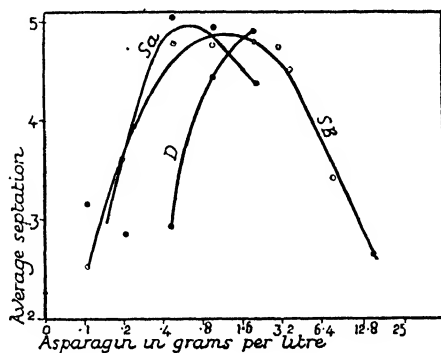


FIG. 13. Graph showing average septation curves for the strains SB, Sa, and D.

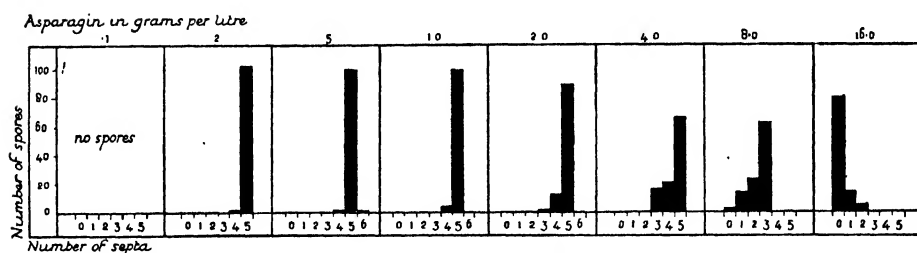


FIG. 14. Graphical representation of the distribution of septation as shown by the strain P^1 .

narrower (0.5–1 A), and the curve slopes more steeply on the left than on the right-hand side.

Strain D. Range 0.1 A–2 A.

With this strain a high 5-mode occurs at 2 A, which is replaced by a high 3-mode at 0.5 A. At lower concentrations spore production ceased. The change in the average septation (Fig. 13) is represented by a curved line which corresponds to the left-hand portion of the more complete curves, shown in the same figure for SB and Sa, but situated farther to the right from the zero point.

Group 2. Strains P, P^1 , and Pp. Of these only P^1 has been investigated.

Strain P^1 . Range 0.1 A–16 A (Figs. 14 and 15).

At 0.2 A, 0.5 A, and 1 A the spores are nearly all 5-septate. The

5-septate spores decrease in number gradually until 4 A is reached. At 8 A the 5-mode is replaced by a moderately high 3-mode. At 16 A 78 per cent. of the spores are 0-septate. The graph for the average septation (Fig. 15) shows the arch and right-hand sloping portion of a curve of the optimum type. The left-hand portion is unrepresented owing to the sudden cessation of spore formation at 0.1 A. The curve for the strain P¹ slopes more steeply than that given in Fig. 13 for SB and almost reaches the base line.

Group 3. Strains CC, CC 1, CB, and CJ (Figs. 16 and 17).

Strain CC. Range 0.1 A–2 A.

A 5-mode occurs throughout the series (Fig. 16). The septation shows a certain amount of irregularity which is probably due to experimental error, since the septation figures in this case were based on counts of 50 spores. The graph for the average septation (Fig. 17) is based on the mean average obtained from the two sets of results (cultures 12 and 19 days old) and shows only the arch of a curve of the optimum type.

Strain CC 1. Range 0.1 A–2 A.

This strain is a saltant derived from the parent strain CC (*Fusarium culmorum*). The results (Figs. 16 and 17, CC 1) correspond to a certain extent with those obtained for Sa (*Fusarium sambucinum*). The saltant differs from the parent in the extent of the fall in the average septation from the optimum in the direction of reduced asparagin concentration (0.1 A).

Strain CB. Range 0.1 A–4 A.

From Fig. 16 it is seen that a 5-mode is present throughout the series, with the exception of concentration 4 A, where the 3's, 4's, and 5's are about equally distributed. A steady decline in the septation from 0.1 A to 4 A is shown in the graph for the average septation (Fig. 17), where only a small portion of an optimum curve is represented.

Strain CJ. Range 0.1 A–16 A.

At 0.1 A and 0.2 A no spores were formed. At 0.5 A and 1 A nearly all the spores were 5-septate. The septation declines to a 3-mode which occurs at 16 A.

The curve for the average septation (Fig. 17) is of similar type to that

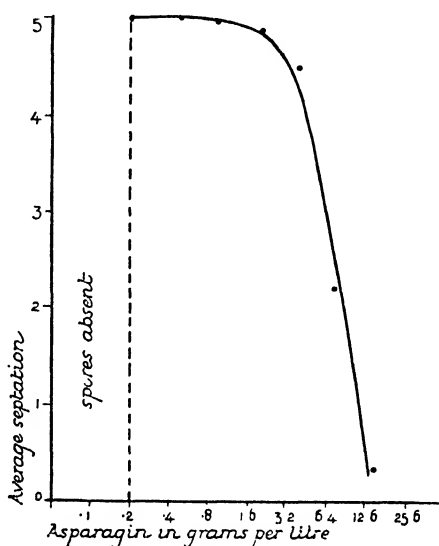


FIG. 15. Graph showing average septation curve for the strain P¹.

shown by CB, but it is truncated on the left through cessation of spore formation at 0.2 A, while the summit of the curve is situated farther to the right.

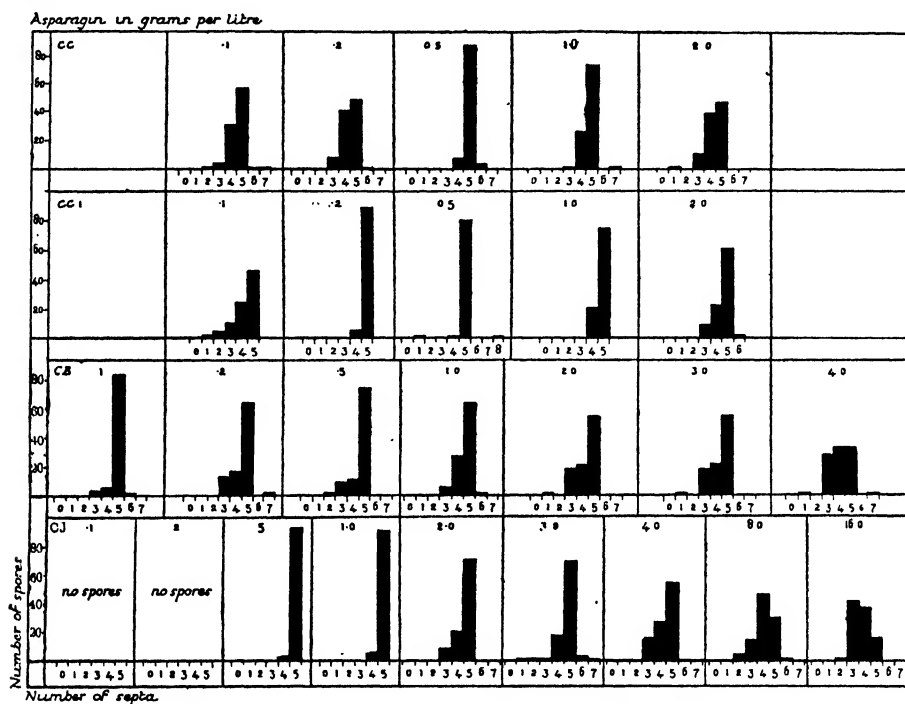


FIG. 16. Graphical representation of the distribution of septation as shown by the strains CC, CC 1, CB, and CJ.

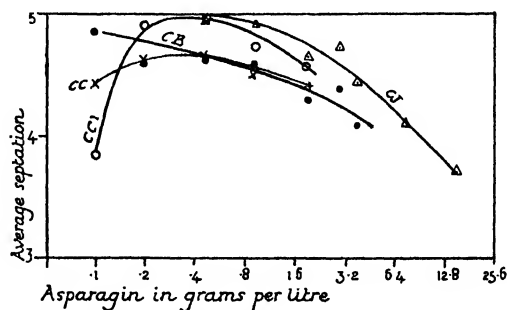


FIG. 17. Graph showing average septation curves for the strains CC, CC 1, CB, and CJ.

Group 4. Strain L (Fusarium lolii).
Strain L. Range 0.1 A–2 A (Fig. 18).

The results are somewhat irregular owing to the fact that the septation counts are based on an examination of only 50 spores. There is, however, a pronounced rise in the septation from 0.2 A, where a 1-mode is present, to

0.5 A, where a high 3-mode occurs. The probable curve for the average septation is shown in Fig. 19; only the left-hand portion of an optimum curve is present.

Group 5. Strain T (*Fusarium trichothecioides*).

Strain T. Range 0.1 A–2 A (Fig. 18).

No spores are present at 0.1 A. A high 1-mode is present at 0.2 A,

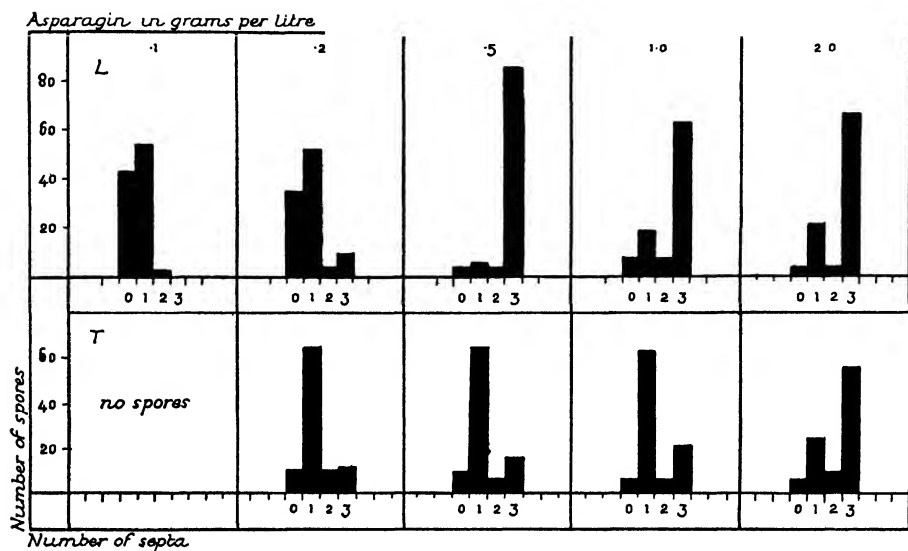


FIG. 18. Graphical representation of the distribution of septation as shown by the strains L and T.

0.5 A, and 1 A. The septation rises at 2 A, at which concentration a 3-mode is present.

The average septation for the series is shown by the curve in Fig. 19. Further work needs to be done with this strain, using a wide range of asparagin concentration before this result can be interpreted satisfactorily.

The effects on spore formation and septation produced by varying the C:N ratio in the cases specified above have been compared with those observed by Brown and Horne for the strains D and A of another species of *Fusarium* studied under approximately similar experimental conditions. For the purpose of the comparison the figures given by Brown and Horne (2, p. 216, Table X) for the strain D 1 (long spore type) have been plotted against concentration of asparagin. The graph (Fig. 20, D 1) shows a curve which is clearly of the optimum type. The figures given in the first column in Table V (2, p. 212) for the strain A have been similarly plotted. In this case (Fig. 20, A), as with the Discolor strains CJ, CB, and P¹, the left-hand downward gradient of the optimum curve is absent. The majority of the strains studied by Brown and Horne showed

incomplete curves of the type obtained for A. Brown and Horne observe that the first two members of the series, viz. no asparagin and 0.05 A, are starvation media as far as nitrogen is concerned, and sporulation is feeble and confined to the neighbourhood of the centre of the colony. The effect of nitrogen starvation is shown in a reduced sporing capacity, but is not reflected in a lowering of the septation. A similar effect is recorded for the Discolor strains CJ and P¹. The same result was obtained for strain CJ

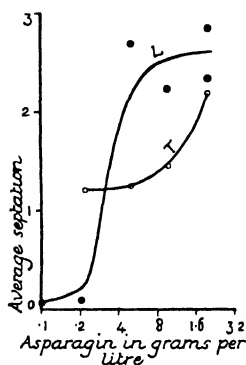


FIG. 19. Graph showing average septation curves for the strains L and T.

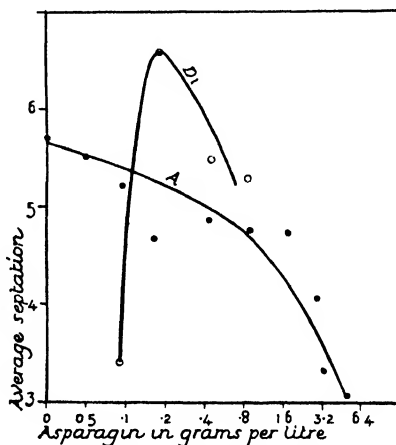


FIG. 20. Graph showing average septation curves for the strains D I and A of the *Fusarium* group investigated by Brown and Horne.

when the concentration of the carbon constituent was increased, the asparagin content remaining constant.

With the majority of the Discolor strains, however, this starvation effect is reflected in the septation, which shows a rapid decline from the maximum in the direction of low asparagin content (see strains CC, CC 1, Sa, SB, T, and L).

Observations as to the effect of the C : N ratio on the nature of the contents of the spores and the development of the atrophied condition are in general agreement with those recorded by Brown and Horne. With most of the Discolor strains the spores were highly granular at the lowest asparagin content (0.1 A), the granularity decreased with increasing asparagin to 0.5 A, granularity was absent and the spores were of the hyaline or vacuolate type at 1 A, and became atrophied at higher concentrations. With regard to atrophy, the strains differed widely as to the amount of atrophy present at a given asparagin concentration after a given time interval. Thus with a certain saltant, CJ 3, all the spores became fully developed and all subsequently showed atrophy associated with a development of sporal chlamydospores within a period of ten days, whereas those of the parent strain (CJ) showed no sign of atrophy during this period of time (on 2 N).

With regard to the development of colour in the substratum, a few strains produced no colour throughout the whole of the asparagin series, others produced little colour, but the majority produced strong coloration, the colours approximating to dahlia carmine, hellebore red, pomegranate purple, and Bordeaux, &c. (Ridgway's colour standards). With all the colour-producing strains the maximum intensity is found between 0.2 A and 2 A. This point is illustrated in Fig. 21, where the relation of colour intensity to altered C : N ratio is shown approximately for the strains CB and Sa.

With the strain CB the colour is most intense at 0.2 A and 0.5 A; it is less intense at 0.1 A, and declines rapidly from 0.5 A in the direction of decreasing C : N ratio to 2 A. With the strain Sa the maximum intensity occurs at 1 A, the intensity lessens towards 0.1 A.

Although the shade of colour is the same at both 0.1 A and 0.2 A the actual quantity of colour is much less at 0.1 A than at 0.2 A. At 2 A the intensity of colour is the same as at 1 A, but at 1 A the substratum is uniformly coloured, whereas at 2 A the colouring is restricted to a peripheral zone about 1 cm. wide, the remaining portion of the substratum being uncoloured. These results are in close agreement with those obtained by Brown and Horne, who found intensity of colouring associated with a high C : N ratio. The weakening in the colouring at 0.1 A (lowest asparagin content) can be ascribed to the feeble growth caused by the limiting concentration of nitrogen.

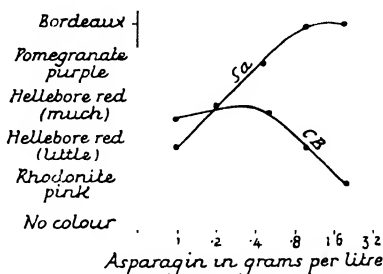


FIG. 21. Graph showing the relation of colour intensity to altered C : N ratio for the strains CB and Sa.

D. EFFECT OF ALTERED CONCENTRATION OF ACID AND ALKALI UPON THE GROWTH-RATE AND SEPTATION.

Series of media were made by adding different amounts of malic acid and sodium carbonate respectively to the standard medium. The complete series was constituted as follows:

Standard medium containing 1.5 per cent. malic acid.

"	"	"	1.0	"	"	"	"
"	"	"	0.5	"	"	"	"
"	"	"	0.1	"	"	"	"
"	"	"	neutral.				
"	"	"	0.1 per cent. sodium carbonate.				
"	"	"	0.5	"	"	"	"
"	"	"	1.0	"	"	"	"

The reactions of the three strains Sa, P, and CC, representative of the groups 1, 2, and 3 respectively, were studied over the complete range of varied acid and alkali under the usual experimental conditions (20° C., light absent). The relation between the growth of the colony and the various members of the acid-alkaline series is shown in Fig. 22, where the actual length of the radius of the colony is plotted against concentration for given time intervals. The graphs given in the figure show a high peak at the neutral point in the case of all three strains. The strains are therefore less tolerant of acid than for example certain species of *Eidamia*, where the

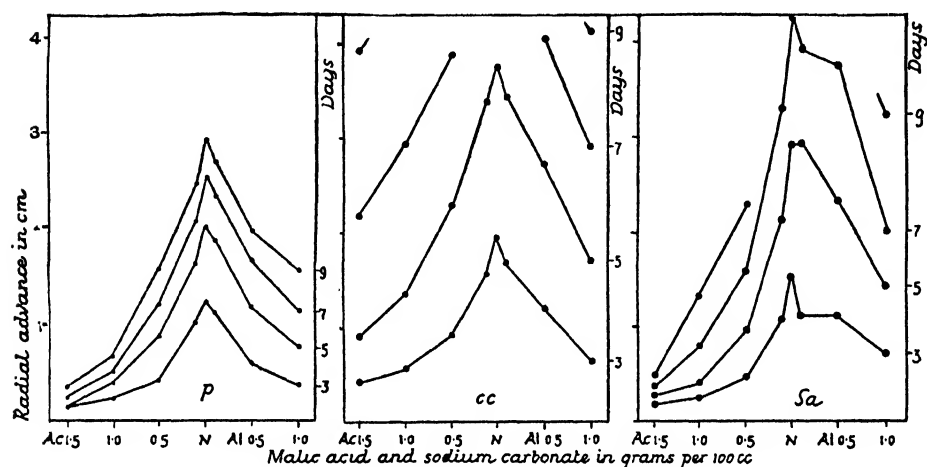


FIG. 22. Acid-alkali series. Graph showing radial advance after different time intervals for the strains P, CC, and Sa.

optimal growth is found on the acid side. The curves shown in the figure are clearly not of the usual optimum type. This is probably due to plotting the actual growth against *percentages* of acid and alkali instead of hydrogen-ion concentrations. Horne and Williamson (4, p. 415) found potato extract agar containing 2 per cent. malic acid equivalent to pH 2.4 and 0.25 per cent. to pH 3. If these figures are only approximately true for the series under consideration the ordinates between 1.5 Ac (1.5 per cent. malic acid) and 0.1 Ac would be situated close to one another, whereas the ordinates from 0.1 Ac and 0.1 Al would be widely separated, since the difference in hydrogen-ion concentration between 0.1 per cent. malic acid and 0.1 per cent. alkali is considerable. It is evident, then, that if the growth were plotted against hydrogen-ion concentration curves of the usual optimum type would be obtained.

It has been found that all the Discolor strains cause the formation of alkali in media containing asparagin as the nitrogen constituent. It was thought therefore that growth would be progressively accelerated in acid media. That this is the case is shown by the graph for the strain CC, given in Fig.

23, where the radial advance is plotted against time for the various members of the acid-alkali series. An examination of the curves shows that acceleration has occurred at 1.5 Ac, 1.0 Ac, and 0.5 Ac, and is most marked at the highest acid concentrations. A similar series of graphs for the staling strain P is given in Fig. 24. This strain is relatively tolerant of acid at 1.5 Ac and 1.0 Ac. The staling character is well shown in the curves for 0.1 Ac, 0.1 Al, and 0.5 Al.

In studying the septation throughout the series of media under consideration it must be remembered that the composition of the media will

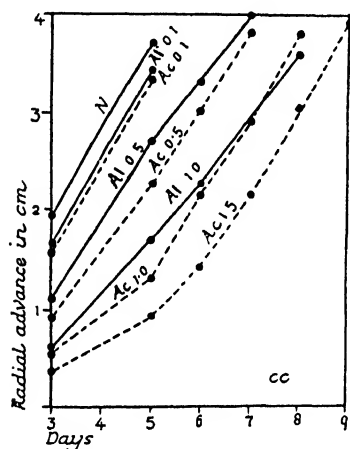


FIG. 23. Acid-alkali series. Graph showing radial advance plotted against time for the strain CC.

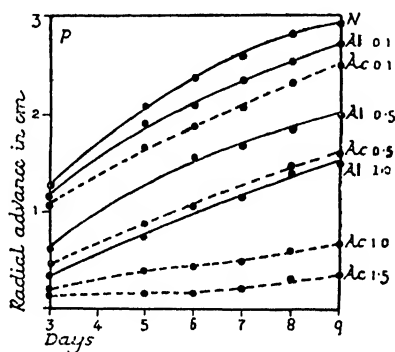


FIG. 24. Acid-alkali series. Graph showing radial advance plotted against time for the strain P.

have changed before the spores attain their full development; that is to say, the alkaline members of the series will have become more strongly alkaline, while the neutral member has developed alkalinity and the acidity of the acid members reduced, neutralized, or changed to alkalinity. If reference be made to Fig. 23 (strain CC) it will be seen that the increment in radial growth between the third and fifth day for N (neutral) is approximately equal to that recorded for 1.0 Ac between the fifth and seventh day and to that for 1.5 Ac between the seventh and ninth day. In other words, the rate of growth at 1.5 Ac after the seventh day is the same as that for N after the third day, from which it may be deduced that 1.5 Ac no longer exhibits acidity in the region of the growing margin of the colony. If this is the case the series must be regarded as one of increasing alkalinity from the point of view of spore septation. The modal change with respect to septation shown by the strains CC, P, and Sa throughout the complete series is represented in Fig. 25. With all three strains a high 5-mode occurs at the acid end of the series, which changes to a 3-mode with increased alkalinity. The results obtained with CC at 1.5 Ac and 0.5 Ac are regarded

as due to experimental error (the septation counts were based on fifty spores). The curves given in Fig. 26 for the same three strains show the probable

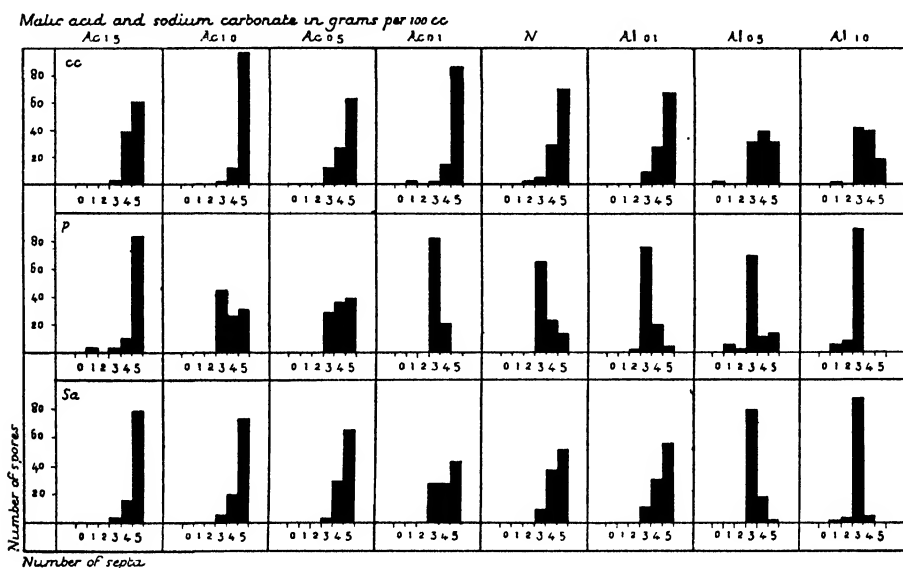


FIG. 25. Acid-alkali series. Graphical representation of the distribution of septation as shown by the strains CC, P, and Sa.

change in the average septation. In every case the right-hand descending portion of an optimum curve is represented. If expressed in terms of hydrogen-ion concentration the curve would be of the same type, but would show a steeper gradient.

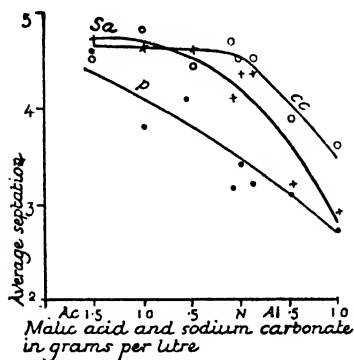


FIG. 26. Acid-alkali series. Graph giving average septation curves for the strains CC, P, and Sa.

The effect on the septation is the same as that obtained on altering the C : N ratio by increasing the asparagin content of the medium. Hence it appears probable that the alkali released as a result of the breaking down of asparagin by the fungus is the principal factor which causes the lowering of the septation.

The colour changes which occur throughout the acid-alkali series are given in Table V. The records were made on the eighth day after the inoculation of the plates. In dealing with the question of colour as with septation the change in the media in the direction of alkalinity must be borne in mind. In fact, on the eighth day, starting from the acid end of each concentration series, the plates will exhibit increasing alkalinity in the

region of the plate occupied by the colony. Hence from the data given in the table, it may be concluded that the maximal colour development and intensity occur in the region of medium alkalinity. That the alkalinity of the medium has some bearing on colour development seems clear from

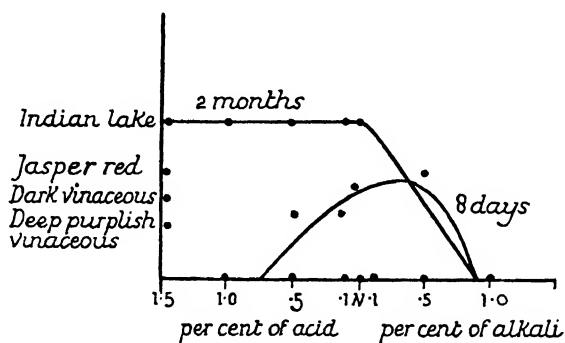


FIG. 27. Acid-alkali series. Graph showing colour development in the case of strain P.

observations made at a later date (two months after inoculation). The results obtained on the two occasions are graphically represented in Fig. 27 (strain P), where the approximate intensity of colour is plotted against initial concentration of acid and alkali. From the graph (Fig. 27) it is clear that the position of maximal colour intensity has passed with time towards the left-hand members of the series, which have become strongly alkaline.

TABLE V.

Medium.	Strain.		
	Sa.	CC.	P.
1.5 Ac	None	Dark vinaceous-fawn zone near centre.	None.
1 Ac	None	Purplish vinaceous . .	None.
0.5 Ac	None	Small yellow centre surrounded by zone of rocelin purple, remaining portion buff pink.	Peripheral deep purplish vinaceous zone.
0.1 Ac	Light vinaceous-fawn peripheral zone.	Buff pink with jasper pink margin.	Similar to 0.5 Ac.
N	Vinaceous-purple peripheral zone.	Buff pink with jasper pink margin.	Similar to 0.5 Ac.
0.1 Al	Centre, colonial buff. Broad peripheral zone of Etruscan red.	Similar to N, but with a little deeper colour.	Substratum dark vinaceous.
0.5 Al	Yellow ochre with narrow peripheral pink zone.	Near Mars yellow and amber brown.	Substratum jasper red.
1.0 Al	None	None	None.

SUMMARY.

1. The strains of *Fusarium*, Section *Discolor*, employed in this investigation, with the exception of those of *Fusarium polymorphum*, show a uniform rate of radial advance when grown in the standard medium (N); that is to say, they are of the non-staling type. The septation mode shown by strains of this type in N varies from 1 (*F. lolii*) to 5 (*F. culmorum*); correspondingly, the average septation varies from about 1.7 to 4.9 (10–13 days). The strains of *F. polymorphum* show a slight retardation in the rate of radial advance in N: the septation mode varies from 3 to 5 (10 days).

2. When the composition of the standard medium (N) is altered, either by varying its concentration or by varying the concentration of the nitrogenous constituent or the hydrogen-ion concentration, the average septation obtaining throughout any series *when the variation in any of the above-mentioned factors covers a sufficiently wide range* may be represented by a curve of the optimum type with gradients from the summit in the direction of high and low concentration. The strains differ from one another in the shape and gradients of the curve and in the position of its summit relative to the scale of concentration. With some strains the curve is abruptly truncated at or near the summit owing to the cessation of spore formation.

3. Certain strains produce no colour in series where the concentration of the nitrogenous constituent is varied; with colour-producing strains the maximal colour development occurs in the region of medium alkalinity.

4. The effect of increasing the glucose constituent of N is reflected both in the capacity for spore production and in the septation. Either the sporangium capacity is reduced or the average septation lowered or both. With *F. culmorum*, strain CJ, spore production gradually falls throughout the range G–20 G, where G represents the initial glucose concentration, but the average septation remains unaltered: with *F. culmorum*, strain CC, both spore production and the average septation decline within this range. In the case of *F. polymorphum*, the septation shows a sharp decline within the range G–8 G. The limit for spore production for the strains of *F. polymorphum* ranges from 4 G to 10 G.

5. Other features associated with an increasing concentration of glucose are as follows: (1) The rate of radial advance tends to diminish; (2) the spores show degenerative changes of various kinds, e. g. atrophy, fragmentation, &c.; (3) the spore contents lose their vacuolate appearance and become more and more granular; (4) colour production occurs within a narrow range of concentration (8 G–10 G); (5) the production of chlamydospores is not obviously affected by altering the concentration.

6. The rate of radial advance for certain representative strains (P, CC, Sa) in media where the acidity and alkalinity is varied reaches a maximum

in the region of neutrality. Such media become alkaline or increasingly alkaline with time, owing to the production of alkali by the fungi concerned as growth proceeds. Hence the colour changes, septation, &c., observed bear no direct relation to the initial concentrations of acid and alkali employed, but show a close correspondence with the changes observed in series where the nitrogenous constituent (asparagin) is varied.

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Growth Studies.

VI. On the Relative Sizes of Growing Plant Organs.

BY

W. H. PEARSALL.

With three Figures in the Text.

A FURTHER analysis of published data (Pearsall, 8) on the correlation between stem growth and root growth has shown that a simple relation exists between the stem weight and the root weight during the periods when both organs are growing. The logarithm of the stem weight (S) is found to be directly proportional to the logarithm of the root weight (R). Expressing this graphically, when $\log. S$ is plotted against $\log. R$, using the results at different times during growth, a straight line is obtained. This relation appears to hold for a great variety of data, from which the following examples have been taken, namely: the growth of shoots and roots in peas, carrots, turnips, cotton, wheat, and barley; lamina and petiole sizes in *Tropaeolum majus*.

Pisum sativum. Values of stem and root weights for etiolated peas have been given in a previous paper (8). One of the three series of results there given (Series IV) is presented graphically in Fig. 1. The other series are essentially similar and do not, therefore, require separate consideration.

In addition, data for the growth of peas in light have been obtained, which are given below. Seedlings of carefully selected initial size were grown in sand moistened from below by Shive's three-salt culture solution. Samples of these (twelve plants in each) were taken at successive seven-day intervals, and the average dry weights of their shoots and roots determined.

TABLE I.

Dry Weights of Pea Plants (in mg.).

<i>Time in Days.</i>	<i>Shoot Weight.</i>	<i>Root Weight,</i>
0	35.0 ± 0.7	7.2 ± 0.4
7	65.7 ± 3.1	14.2 ± 1.1
14	152 ± 4.7	33.4 ± 2.5
21	303 ± 8.4	68.7 ± 1.9
28	502 ± 11.7	116.0 ± 4.2

These data are represented graphically in Fig. 1.

Plants with fleshy roots. Results obtained for the relative sizes of turnip shoots and roots are given for comparison. These figures are average

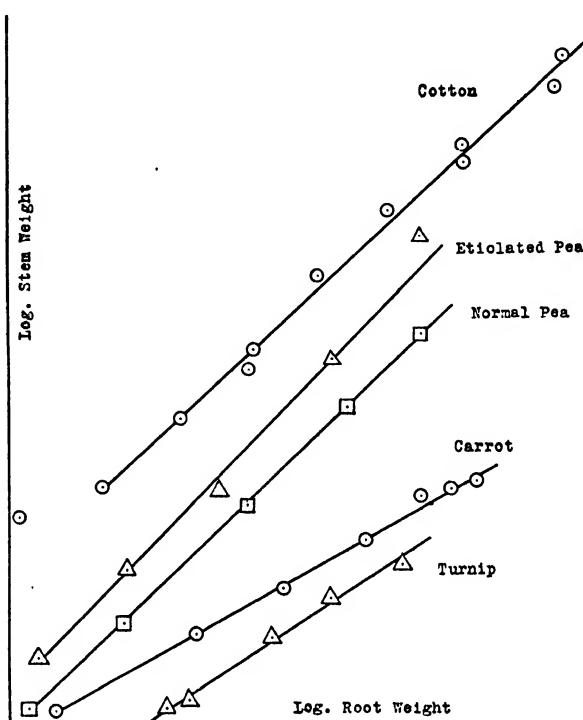


FIG. 1. Relation of logarithms of stem weights and root weights for various plants. The vertical scale is reduced to one-half in the case of etiolated peas.

fresh weights of material grown in loose soil in the open. The averages are for twenty plants. The roots were carefully removed, washed with a fine jet of water, and dried on filter-paper. The plants had been initially carefully thinned, so that the initial plants were all of similar stem size.

FRESH WEIGHTS OF ROOTS AND SHOOTS OF *BRASSICA RAPA*, *RAPIFERA* IN GRAMMES.

Time in Days.	Stem Weight (S).	Root Weight (R).
0	3.94	0.20
5	4.35	0.26
10	8.9	0.70
15	14.15	1.40
20	21.0	3.14

The relationship between log. *S* and log. *R* is given graphically in Fig. 1, along with the logarithmic values of the stems and roots of carrots (*Daucus carota*) as recorded by Deleano (3).

Other plants. Inamdar, Singh, and Pande (6) have recorded the rela-

tive sizes of shoots and roots for cotton grown in India. The data for Series I of their paper are given graphically in Fig. 1. Reference to the original paper will show that similar relationships are found in their other series of results for cotton and balsam.

In all of these cases it is evident that $\log. S$ is directly proportional to $\log. R$. This result holds equally well for other data examined. For example, in the case of cereals, it is shown by the data of Weaver, Kramer, and Reed (12) for winter wheat comparing photosynthetic area and root area; for barley by the data of Turner (11) and Newton (7) comparing dry weights.

Measurements of an entirely different character have been made on the leaves of *Tropaeolum majus*. The average diameter of the lamina and the length of the petiole were measured on a series of growing leaves (I) and on all the leaves of a large plant growing in the shade (II).

LINEAR DIMENSIONS ON *TROPAEOLUM* LEAVES (IN CM.).

Series I.		Series II.			
Lamina Diameter.	Petiole Length	Lamina Diameter.	Petiole Length.	Lamina Diameter.	Petiole Length.
1.2	0.9	2.2	1.5	6.5	15.0
1.6	1.3	3.0	2.9	7.1	15.9
2.3	2.0	3.4	4.9	7.0	15.3
2.8	2.7	3.2	5.0	6.9	16.0
4.0	3.9	4.0	6.1	7.3	18.0
5.1	5.1	4.8	8.4	6.8	16.8
6.4	6.9	5.0	9.1	8.2	21.0
—	—	5.4	10.9	10.0	28.1
—	—	5.9	11.5	9.5	34.4

The graphical relation of the logarithms of lamina diameters to the logarithms of petiole lengths is given in Fig. 2. It is obvious that here also these two quantities give a straight line.

We may assume, therefore, that the relationship between the sizes of two growing plant organs is such that the logarithm of the size of one organ is directly proportional to the logarithm of the size of the other organ. Brief reflection serves to show that this is a logical result of the fact that the growth rate of plants and of plant organs is logarithmic in character (1, 9). Thus, comparing the sizes of shoot (S) and root (R) at the same time interval (t) after growth has started, we may say that $S_t = S_0 e^{xt}$, where S_t is the weight of the shoot at time t , S_0 its initial weight, x is the average logarithmic growth rate of the shoot, and e the base of the natural logarithms (cf. Blackman, 1). Similarly, the weight of the root would be $R_t = R_0 e^{yt}$, using a similar notation with y as the average logarithmic growth rate of the root.

Since the initial shoot weight must bear a definite ratio (c) to the initial root weight, $S_0 = cR_0$.

The values S_t , R_t , S_0 , and R_0 are all functions of R_0 , so that the latter may be taken as the unit. If, then, $R_0 = 1$ $S_t = C.e^{xt}$ and $R_t = e^{yt}$ or $\log. R_t = yt$,

since $t = \frac{\log. R_t}{y}$ and $\log. S_t = \log. c + xt$, substituting for t

$$\log. S_t = \log. c + \frac{x}{y} \log. R_t$$

or $S_t = cR_t^{\frac{x}{y}}$, in general $S = cR^k$,*

where c is a constant of the relative initial sizes of S and R and $k (= \frac{x}{y})$ is the ratio of the average logarithmic growth rates. When the values of $\log. S$ and $\log. R$ in this equation are plotted against one another, a straight line will obviously result.

The value of k may be derived from experimental results, either by calculation or more simply by determining the slope of the straight line from the graph. The tangent of the angle made by this line with the x ordinate gives the value of k . (The value of c is less easily derived. The initial weight of the shoot or root must be known before this can be done.) The approximate values of k for the shoot-root ratio have been obtained for a number of plants. They are given in the following table:

APPROXIMATE VALUES OF k FOR SHOOTS AND ROOTS.

Plant.	k .	Data from:
<i>Pisum sativum</i> , etiolated	1.75	Pearsall (8), Series III.
" " "	2.20	" " IV.
" " "	2.65	" " V.
" " in light	0.95 s	See text.
" " "	± 0.90	Brenchley ¹ (2).
" " "	1.15	Newton ² (7).
<i>Hordeum distichum</i>	1.15	" ³ (7).
<i>Triticum vulgare</i>	1.05 s	Weaver, Kramer, and Reed (12).
<i>Hordeum distichum</i> , low N	1.20	Turner (11).
" " high N	1.55	"
<i>Linum usitatissimum</i>	1.30	"
<i>Gossypium roseum</i>	0.90 s	Inamdar, Singh, and Pande (6).
<i>Impatiens</i> sp.	1.00 s	" " " "
<i>Lupinus albus</i>	± 1.15 s	Gain ³ (4).
<i>Daucus carota</i>	0.55 s	Deleano (3).
<i>Brassica rapa</i>	0.65 s	See text.

s = soil cultures.

* If R_0 is not eliminated, the equation is $S_t = cR_0 \left(\frac{R_t}{R_0}\right)^{\frac{x}{y}}$.

If t is not eliminated, it becomes $S_t = cR_0 e^{(x-y)t}$.

These alternative forms are occasionally useful.

¹ Measurements of 3rd, 4th, and 5th weeks, 1916.

² Grown in same solution.

³ Growth of plants intermittent.

It will be seen from these figures that the value of k fluctuates considerably. Some of this fluctuation is probably due to the genetic differences of the plants given in the table. Thus both the species with fleshy roots give low values of k . On the other hand, the conditions of growth clearly exert an influence. This is seen by comparison of the figures for barley, high and low nitrogen. High N clearly increases the relative efficiency of the stem. Further, etiolation also greatly increases the value

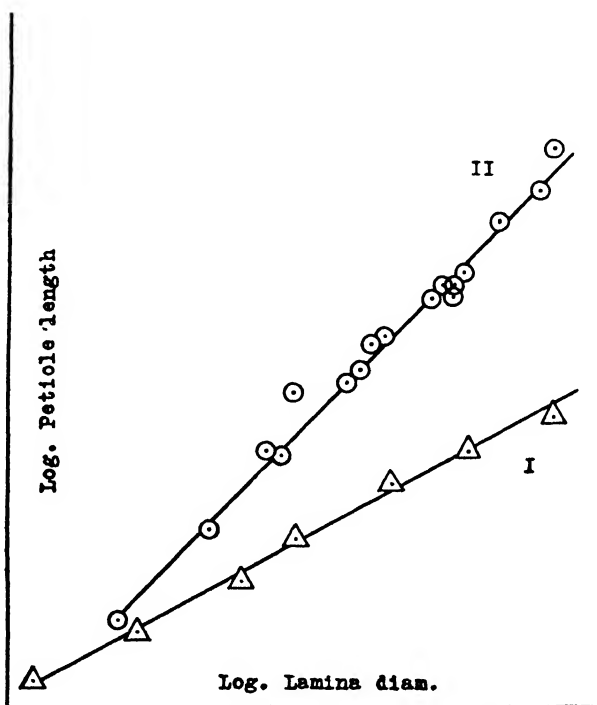


FIG. 2. Relation between logarithms of lamina diameters and petiole lengths of *Tropaeolum majus* leaves. I. A series of growing leaves. II. From all the leaves of a large plant growing in the shade.

of k in *Pisum*. Finally, when two species, peas and barley, are grown in the same culture medium, the value of k is the same for each. In part, then, the variations in k are clearly due to the influence of environment. To this influence we are inclined to ascribe the fact that the soil cultures give rather lower values of k than water cultures. There is always a danger of retardation of root growth in water cultures, and while this might be slight and not noticeable in the cultures, it would undoubtedly tend to reduce the values of root weight and hence to give high values of k . The soil cultures are, therefore, more reliable on the whole. Emphasis is laid upon this point because the values of k clearly range round unity, especially in the case of the soil cultures.

In order to see what possible interpretations may be placed on the values of k , let us assume that k is normally one (for the shoot-root ratio). Since k is the ratio of the relative logarithmic growth rate of the shoot and root, the fact that it may normally be unity indicates the probability that shoot and root may possess the same logarithmic growth rates, or, using the suggestion of Blackman (1), that they may have similar growth efficiencies. If this were so, under common conditions of growth a single dividing cell from the shoot meristem would produce the same number of cells in a given time as a single dividing cell from the root meristem. Since both shoot and

root cells were originally derived from the same protoplasm, there are clearly some grounds for this supposition. The relative logarithmic efficiencies of the two growing points need not, however, depend entirely on the relative efficiencies of the constituent cells. Spatial relationships may also produce effects. In order to illustrate this point we may consider the following somewhat crude scheme.

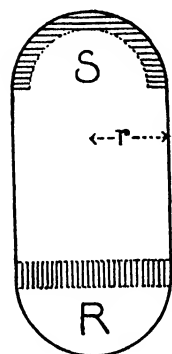


FIG. 3. Diagrammatic representation of stem and root.

The embryo may be represented diagrammatically (Fig. 3) as a spindle. Since the root grows endogenously and the shoot superficially (cf. Schuepp, 10), then we may assume for the purpose of argument that the relative growing points will be represented (*a*) by the circular cross-section of the root, multiplied by some quantity to indicate the linear thickness of the meristem, (*b*) by the approximately semi-spherical surface of the stem, multiplied by a quantity representing the depth of this meristem. In a given time, therefore, the amount of material produced by the root, R , will be proportional to the area of the cross-section, i.e. to its radius squared or r^2 , and its initial size and depth. Similarly the amount produced by the stem, S , will be proportional to the surface of a hemisphere, i.e. to r^2 , and the initial size and depth of the stem meristem. Let c represent the extent to which S is greater and/or thicker than R .

$$\begin{aligned} \text{Thus, if } R &\text{ is proportional to } r^2, \\ S &\text{ „ „ „ to } cr^2, \\ \text{whence } \log. S &= 2 \log. r + \log. c, \\ \log. R &= 2 \log. r, \\ \text{and (1) } \log. S &= \log. R + \log. c. \end{aligned}$$

In this statement two assumptions are made. First, that the growth of either stem or root may be considered as taking place over an area proportional to the diameter or radius of the spindle; secondly, that the cells of both organs are growing at the same logarithmic rate. To correct, if the latter possibility is not the case, it is necessary, as in the first deductions, to multiply $\log. R$ by a factor representing the ratio between the relative

logarithmic growth rates. Assuming that these rates are the same, however, we can proceed to consider the first of the above assumptions.

If the whole of the semisphere at the stem apex were growing, then the growth of the stem would be proportional to the volume of the semisphere, i.e. to r^3 , and the equation would become

$$(2) \log. S = 3/2 \log. R + \log. c.$$

Conversely, if the whole of the volume of the root apex were growing, while the stem was growing superficially, then

$$(3) \log. S = 2/3 \log. R + \log. c.$$

Generalizing these become

$$(4) \log. S = k \log. R + \log. c,$$

where k may be a quantity (*a*) of value 1 if both stem and root are growing, so to speak, as areas, or, if both are growing as volumes, (*b*) of value 1.5 when the stem is growing as a volume and the root as an area, (*c*) of value 0.66 when the stem grows as an area and the root as a volume. This somewhat crude method of considering the problem illustrates clearly that differences in the values of k for different plants may be due not only to the relative efficiencies in cell division of the individual cells in stems and roots, but also to whether the zone of dividing cells is functionally chiefly a volume or an area. It is clear that these spatial conditions will affect the values of k obtained in some cases. Thus in carrots and turnips the values of k are 0.55 and 0.65—very near to the ideal value of 0.66, which would indicate that the shoot was growing as an area and the root as a volume. The agreement in this special case appears to be too suggestive to be lightly dismissed. The high values of k , 1.75 to 2.65, found in etiolated peas may be due to the reverse condition. The apical meristem of the shoot in etiolated peas is not merely superficial as in most flowering plants, but it occupies the whole volume of the stem apex, giving rise to a plumular hook composed of meristematic cells. The growth of such a tissue should tend to be a function of its volume, and hence the value of k should theoretically be 1.5. The fact that the values of k actually obtained are higher may be due to an increased logarithmic rate of growth of the stem apex in the dark, or perhaps more possibly to the fact that the data are derived from water cultures in which the probability of retardation of root growth has always to be considered.

In conclusion, it is not desired to extend these arguments too far in this preliminary inquiry, so much as to indicate the fact that the relative sizes of plant organs may be readily expressed in terms of the classical laws of plant growth. The equation derived to express the interrelationships is free from the disadvantages of many mathematical incursions into biology, in that every term employed has been shown to possess a biological

meaning. It is probable that the law here formulated is of general application, since Huxley (5), in a preliminary note, has recorded a similar relation between the size of an organ and that of the body in some animals.

SUMMARY.

The relative sizes of plant organs are shown to conform to the equation $x = cy^k$, where x and y are the sizes of the organs, c is a constant expressing their relative initial sizes, and k is a quantity expressing the ratio of their relative logarithmic growth rates and the spatial arrangements of their meristems.

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On the Germination of the Seed of *Spinacia oleracea*, L., at Low Temperatures.

BY

H. B. SIFTON.

With Plate XXV and five Figures in the Text.

WHILE conducting germination tests in the Canadian Government seed laboratories, my attention was attracted to the fact that spinach seed almost invariably gave a lower percentage germination than the appearance of the samples seemed to warrant. A number of seeds, presenting no difference in appearance from those that produced strong plants, would fail to germinate, and this occurred even in samples not exhibiting the slow or irregular germination characteristic of weakened seed. Many different conditions of germination were experimented with in an effort to ascertain whether the individuals that failed to grow were really dead, or merely unable to germinate under the accepted method of testing, i.e. the use of a standard germinator maintained at alternating temperatures of 18° C. and 20° C.

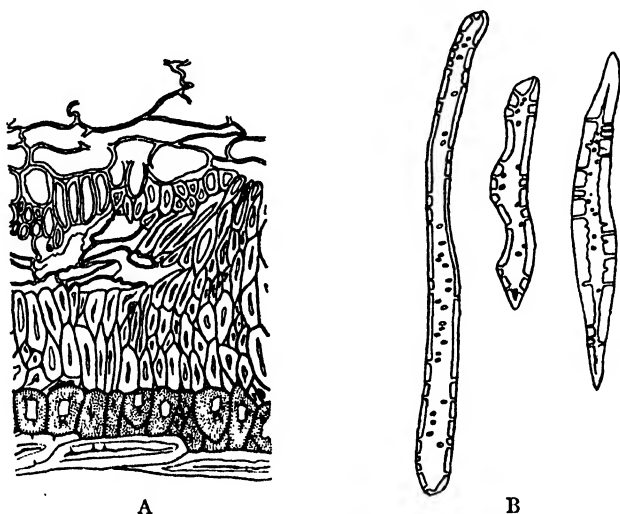
It was found that by allowing the water from melting ice to drip continuously on the seeds, the germination percentage was considerably increased, though the time required was much lengthened. The temperatures of the two sets of tests are distinct and easy to maintain, a variation of three or four degrees in either making no appreciable difference in germination, and the difference in results is significant. For this reason spinach seed was chosen as a basis of investigation into the favourable effect of low temperature on germination.

The usually accepted place of origin of spinach, somewhere in south-western Asia, leaves us with a considerable range of climatic conditions under which the species may have grown originally. Unless an important change has occurred under cultivation, however, we should expect that the region would be one of coolness and moisture during the vegetative season, for spinach is recognized as a cool weather plant, going to seed with the coming of summer heat.

The complete fruit is harvested and sold for seeding purposes. That

of the smooth-fruited variety, which has been used in this investigation, is illustrated in Pl. XXV, Fig. 1. It is a heart-shaped structure, slightly flattened, the point of attachment to the plant being at the apex. At the other end may be seen a protuberance, the base of the style.

Pl. XXV, Fig. 2, shows the fruit with one half of the pericarp removed, exposing the seed within. The ovary wall is seen to be fairly thick. Text-fig. 1 illustrates its structure. Of this figure, drawing (A) is from a cross-section, the outer part of the ovary being towards the top. On the outside is a region of parenchyma, with the outer cells broken and some of



TEXT-FIG. 1. Pericarp of spinach fruit. A. Transverse section. $\times 160$. Outer region of pericarp uppermost. B. Types of lignified fibre. $\times 229$. From a maceration.

them worn away. Then comes a rather thick layer composed of lignified fibres. The three types of fibre are illustrated from a maceration in Text-fig. 1, B. Through this fibrous layer, extending from the point of attachment, are small fibro-vascular bundles, and in the region of these the thinner-walled fibres are congregated. All the fibres are copiously pitted, and they form a continuous layer except at the point of attachment, where a vascular bundle enters to supply the seed, and in the region just below the style, where there is a narrow rod of thinner-walled cells extending through the pericarp. Proceeding inward, a single layer of heavy-walled stone cells is met. These also are pitted, and many of them contain crystals. The walls of these cells are so thickened that when no crystal is present the lumen is almost occluded. The lining layer of the pericarp consists of fibres whose walls have not become lignified.

Returning to Pl. XXV, Fig. 2, it will be seen that the seed lies more or less loosely within the ovary. In some cases there is an easily discernible space between the seed and the ovary wall. At the point of attachment to

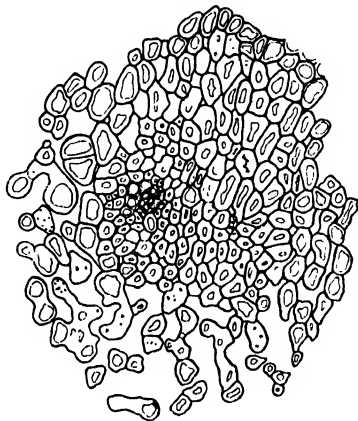
the plant the ovary wall extends inward, forming a broad column which connects it with the seed, and may be seen in Pl. XXV, Figs. 2 and 4. A section across this column is shown in Text-fig. 2. Near the centre is the fibro-vascular bundle passing in towards the seed, surrounded by tightly packed fibres similar to the lignified ones of Text-fig. 1, B. Outside of these, shown to the lower left of the figure, is a spongy mass of loose cells with many air-spaces. This column connects with the seed through the hilum, which is shown clearly in Pl. XXV, Fig. 3, a photograph of the seed removed from the ovary.

The brown seed-coat, of which a section is shown in Text-fig. 3, is composed of several layers of empty cells, more or less collapsed, with a lining of a single layer of cells filled with brown contents. It is very thin, its thickness, as may be seen from the drawing, varying from about 14μ to 25μ , depending on the degree of collapse suffered by the cells.

In Pl. XXV, Fig. 4, the fruit and seed have been cut across. The centre of the seed is filled with a starchy endosperm, about which the embryo is coiled in a single loop, the radicle tip meeting the ends of the cotyledons beneath the hilum.

Three samples of seed of different origin were purchased for germination experiments, and later the work was checked by tests on two more samples of another year's growth. Germination tests were done in duplicate, and the experiments were repeated, in most cases several times, and always with consistent results.

In all germination tests Canton flannel was used as a substratum, the cloth being folded so that two layers were on each side of the seed. Two hundred seeds were used for each duplicate test. Low temperature tests were carried on in a metal can such as is used for shipping ice-cream, which was kept in a tub surrounded with chopped ice. In order that the can might be covered with ice, it was necessarily kept closed, and no ventilation was provided except when the seeds were removed for counting or for temperature determination. A wire rack was made to fit into the can, and on this the samples were supported one above the other, at a distance apart of approximately one and a half inches. The cloths containing the seeds were spread on pieces of wire screen of one-fourth inch mesh, so that air was available from both sides. Temperatures taken twice daily by inserting a thermometer between the layers of cloth showed that



TEXT-FIG. 2. Transverse section of *Funiculus*. $\times 215$.

seeds near the bottom of the apparatus were maintained at a temperature of about one degree centigrade. Sometimes it rose to two degrees, and on three occasions when the ice ran low it rose as high as four degrees. Several times in cold weather the temperature sank to zero or slightly below, but this temperature produced no observable effect on the germination. The temperature of samples at the top was in general from one to two degrees higher than that at the bottom. The duplicate samples were always placed one in the upper and one in the lower part of the apparatus, and the difference in temperature produced no difference in percentage germination.

For tests at room temperature bell-jars were supported above water in crystallizing dishes, a free space for the entrance of air being maintained



TEXT-FIG. 3. Section through seed-coat. $\times 625$.

between the water-surface and the rim of the bell-jar. Samples were supported under bell-jars on wire screening of the same type as that described above. The temperature of the room varied somewhat, being generally between seventeen and twenty-two degrees centigrade, but sometimes exceeding these limits.

The germination percentages of the three samples first experimented with under these conditions were as follows:

<i>Condition.</i>	<i>Sample I.</i>	<i>Sample II.</i>	<i>Sample III.</i>
Room temperature	72	76	73
Low ,,	79	84	83

A significantly higher percentage germination at the lower temperature is evident in each sample, and the results were confirmed by repeated retests.

To determine whether the preference for low temperatures was due to properties inherent in the germ itself or to some action of the enveloping structures, tests were set up with the latter partially cut away. With a small piece removed from the pericarp of each seed, the seed-coat being untouched, the percentage germination was as follows: Sample I, 82; Sample II, 81; Sample III, 81. Although this test was made in June and the temperature of the room was, on the average, somewhat higher than when the former tests were performed, the results correspond to those obtained by the use of low temperatures. A set of tests with a portion of the seed-coat also cut away gave no indication of any additional advantage. In fact, results under these conditions had a tendency to be somewhat lower and

more irregular, due apparently to the difficulty of removing the seed-coat without injury to the embryo directly beneath it.

The specific action of the pericarp was next to be investigated, and three possibilities presented themselves. The ovary wall might, by its physical strength, prevent the developing embryo from forcing its way out, the excretion of waste products might be prevented or hampered, or some substance from outside necessary for germination might be wholly or partially excluded.

As may be surmised from the description of the pericarp structure, water was able to enter all the seeds to the full extent of their requirements. The experiment which provided the solution was suggested when, in order to save space, some seeds were put to test in covered Petri dishes at room temperature, and germinated so poorly that they were removed before the test was complete. This suggested the possibility that the detrimental action of the pericarp consisted in hampering either the entrance of oxygen or the exit of carbon dioxide. The truth of the former alternative was tested by placing samples for germination under an increased oxygen pressure. The bell-jar which covered these samples was lowered until its mouth dipped in the water, and pure oxygen produced electrolytically was admitted at the top in a slow but continuous stream, passing out under the rim at the bottom through a bent glass tube. The germination percentages under these conditions were: No. I, 79; No. II, 85; No. III, 80. These results, which also correspond to those listed above as resulting from the use of low temperature, have, as before, been confirmed by several tests, all of which agree well within the limits of variation to be expected in seeds chosen indiscriminately from the well-mixed samples.

A fourth method of obtaining the higher percentage of germination may be mentioned. As noted above, a certain percentage of the seeds fit loosely in the pericarp with a space between them and its inner wall. This space remains filled with air even when the seeds are soaked. Even those which fill the ovary have a pocket of air about the micropyle and hilum, enmeshed in the spongy parenchyma of the funiculus. When respiration is increased at the beginning of germination this air must become deficient in oxygen and charged with carbon dioxide. In samples that were germinated in air at room temperature and in which the air in this space was changed twice a day by exhaustion under an air-pump, the following results were obtained: No. I, 78 per cent.; No. II, 87 per cent.; No. III, 88 per cent. In the case of Nos. II and III this treatment gave a result slightly higher than that produced in any other way.

As stated already, two more samples of seed were used to check the results obtained. One of these was a poor sample, and the other exceptionally strong. The germination results obtained in these check tests are as follows:

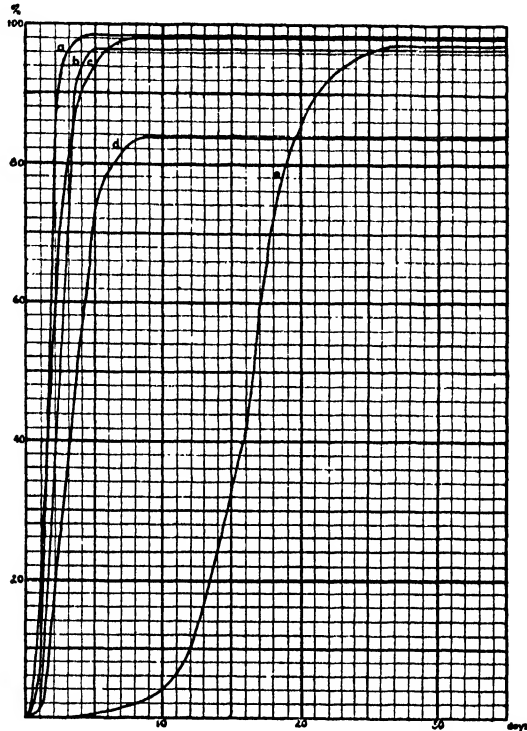
<i>Condition.</i>	<i>Percentage Germination.</i>	
	<i>Sample IV.</i>	<i>Sample V.</i>
Room temperature in air	52	84
Low temperature in air	46	97
Room temperature, pericarp cut	66	99
In atmosphere of pure oxygen	71	97
Room temperature, air exhausted twice daily	64	98

Sample V gave results corresponding in every respect to those of the first three samples, whereas Sample IV germinated poorly at the low temperature. In the living seeds of this sample, low vitality was indicated clearly throughout the tests, and it is apparent that the strength of some of the seeds was not sufficient to withstand the depressing effect exerted by low temperature. This depressing effect is illustrated in Text-fig. 4, which has been included to provide a comparison of the germination curves of a sample under different test conditions. The curves are drawn from a set of duplicate tests made on seeds from Sample V. Counts were made every day, and from the curves may be read the percentage germination at any time during the test. Curve *e*, representing the germination at the low temperature, gives an indication of the depressing action of cold as shown in one of its results, the slowing down of the germination processes. The strongest seed has not completed the changes preliminary to bursting through its enveloping coats until some time between the fourth and the fifth day of the test, and the least vigorous has reached this stage only after twenty-seven days, whereas under all the other sets of conditions the first seed has sprouted within one day of the beginning, and germination is complete in from five to nine days. The behaviour of Sample IV strengthens a suspicion uncontradicted by any results obtained from the other samples, namely, that our lower temperature is not the optimum one for the germination of unclipped spinach fruits at the ordinary oxygen pressure. It is probable that investigation would indicate a temperature between the two extremes used in these experiments, at which samples of ordinary strength would germinate as well as, and more rapidly than, at the lower temperature used here.

Two points of significance have been proved by the germination experiments, namely, that normal spinach seed in its natural condition does not germinate as well at room temperature as at a temperature considerably lower, and that the disability under the former condition can be removed by cutting the pericarp, increasing the oxygen pressure, or providing for a change of air within the pericarp at frequent intervals. It remains to consider what connexion, if any, underlies these results.

Kidd (2), working with several species of seeds, has found that carbon dioxide has an inhibiting effect on their germination, and the possibility of a similar action in this case may be considered. At higher temperatures

oxidation is naturally more rapid, and one could assume, at least during the earlier stages of germination, a greater concentration of carbon dioxide within the pericarp. The concentration would be relieved by cutting the pericarp or ventilating its interior. Moreover, either of them, and, as well,



TEXT-FIG. 4. Germination curves of spinach seed under various conditions. Abscissae = time in days from beginning of experiment; ordinates = percentage of seed germinated. Curve (a). Fruits with pericarp cut, maintained at room temperature in air. Curve (b). Complete fruits at 4° C. above room temperature in oxygen. Curve (c). Complete fruits at room temperature in air; air exhausted from seeds twice daily and replaced by a fresh supply. Curve (d). Complete fruits at room temperature in air. Curve (e). Complete fruits, surrounded by chopped ice during germination period.

the carrying on of the test in pure oxygen, would result in a more plentiful supply of the latter gas, which Kidd found efficient in counteracting the effect of carbon dioxide. There are reasons, however, for believing that in the case of spinach other factors than concentration of carbon dioxide are concerned. In his experiments, Kidd found that the inhibiting effect of carbon dioxide increased, while the stimulating effect of oxygen decreased, with a lowering of temperature. Spinach seed, on the contrary, germinated to better advantage at low temperatures even when placed in a closed vessel entirely without ventilation except when the lid was removed for seed counts or temperature determinations. Granting slow oxidation under these conditions, it would still seem that within the four to five weeks

necessary to complete the test the carbon dioxide collected must be able to produce an inhibiting effect under the favourable temperature conditions, if, indeed, the effect obtained at higher temperatures were due to a concentration of this gas in the ventilated germinating chambers.

The well-known facts concerning fermentation and other analogous reactions in living bodies furnish a possible explanation of the results. During many anaerobic fermentations, as well as during the metabolism of sugar in such forms as the succulents, various organic acids and other products accumulate. In muscle stimulated to such an extent that the oxygen supply is insufficient, lactic acid accumulates in an analogous manner. The spinach embryos stimulated by the higher temperature, and with their oxygen supply hampered by the thick pericarp, are placed under identical conditions, and it is conceivable that they may be producing a substance that, when it becomes sufficiently concentrated, poisons them and prevents the completion of germination.

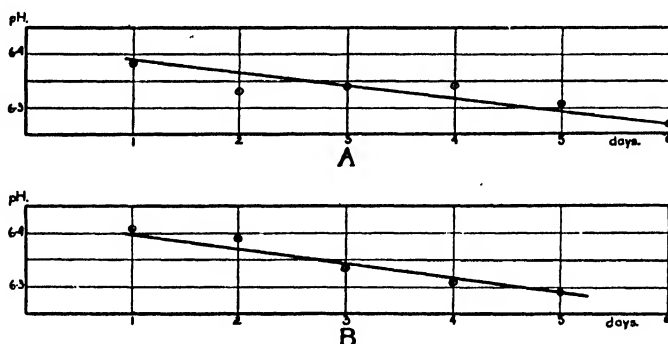
Many of the known products of such reactions are acid, and tests made by cutting individual seeds and applying indicators of the Clark and Lubbs series to the cut surfaces indicated little, if any, lowering of pH during the germination of seeds packed in ice, but a considerable change in those germinated at higher temperatures. These results were checked by the method described by Brown (1) for determining the pH of small quantities of material. The glass cells used contain six to seven drops of water. In practice a seed was bisected with a clean knife, the germ dissected out with clean needles, and crushed and macerated in freshly boiled, redistilled water in one of the cells. The cell was then filled with the water, a drop of indicator added, and the pH determined. For crushing the embryo a small pestle was used consisting of a drawn-out glass tube, on the end of which a bead was formed in the flame and flattened. The limitations of the colorimetric method preclude the possibility of precise measurement of the small change in pH from one day to the next, but the presence of such a change and its progressive character are shown clearly, and the results are sufficiently accurate for our purpose.

Most of the tests for hydrogen-ion concentration were made on seeds tested at the higher temperatures in air and in pure oxygen. Sufficient work on seeds germinating in ice was done, however, to indicate clearly that the pH did not become lowered to a point approaching that at which germination stops when the test is carried on in air at room temperature. Of all the seeds tested from the ice-box only one registered a pH of less than 6.3, and this reading occurred in testing a sample that had been under germinating conditions for thirty-three days.

At the higher temperatures there was a noticeable and gradual lowering of pH throughout the test. The rate of decrease was not more rapid in air than in pure oxygen. In pure oxygen, however, the rate of germina-

tion was always increased, either due to stimulation by the oxygen, or to the fact that the oxygen-filled chamber was kept at a temperature three to four degrees higher than room temperature, owing to the heat produced during electrolysis.

The curves in Text-fig. 5 are obtained from Sample V. Seeds from this sample were placed in the germinating chambers, and each day ten seeds that had not yet germinated were removed from each test and their



TEXT-FIG. 5. Curves showing the lowering of pH in spinach embryos during the germination period, the pericarp being uninjured. A. At room temperature in air. B. $4^{\circ}\text{C}.$ above room temperature in oxygen. Abscissae = time in days from beginning of experiment; ordinates = pH of embryos.

embryos tested individually for hydrogen-ion concentration. The points marked on the curves record the average pH for the day under each of the two conditions.

During the experiment the results from six individual germs indicated clearly, by their divergence from the average, the existence of an abnormality or a mistake. These embryos were excluded from the calculations.

The curves indicate a consistent lowering of pH, a rise in hydrogen-ion concentration. When they are considered in connexion with Text-fig. 4, it is seen that the germination curve of the air-germinated seed begins to fall off at the end of five days, or when, as shown in Text-fig. 5, the average pH produced in the cell of water by the germ of a seed is about 6.3. The seeds germinating in pure oxygen had lowered their pH by an equal or slightly greater amount in the same time, but their germination curve had begun to fall off before the fourth day, owing to almost complete germination, and those tested on the fifth day belonged to the $3\frac{1}{2}$ per cent. that failed to germinate. The result is thus in harmony with the hypothesis the experiment was designed to test. The shape of the curves in Text-fig. 4 also adds confirmation to the theory, the gradual flattening of the curve *d*, between the fifth and ninth day, suggesting the accumulation of some inhibitory substance in the seeds. Similar experiments with others of the samples confirmed the conclusions just stated, the only difference being

a lack of smoothness in the pH curves, owing, presumably, to the presence of a larger percentage of dead seeds, which could not be distinguished and excluded from the tests.

It is probable that the cessation of germination is not due to the increase in acidity, but to the accumulation of some poisonous substance, of which the acidity increase may be an indication. It is clear that such an hydrogen-ion concentration is not, in itself, harmful to spinach, for at least the tips and piliferous areas of growing roots attain an acidity much more pronounced.

The fact is not forgotten that the germinating seeds are constantly producing carbon dioxide, and that water will hold enough of this gas in solution to produce a hydrogen-ion concentration as great as that shown in the curve. It seems hardly likely, however, that the comparatively small embryo would contain enough carbon dioxide to raise the concentration to this extent in the amount of water used for the tests. If it did so, a greater lowering of pH should have been found for the seeds germinated at low temperature. The cold water in their embryos might, in fact, be expected to hold more carbon dioxide in solution because of its low temperature. Moreover, a series of tests made with hot water in cells freshly removed from boiling water, and with the pestle also freshly boiled, produced a similar result of hydrogen-ion concentration rising as the time in the germination chamber increased, a result not explainable on the basis of carbon dioxide dissolved in the hot water.

The possibility of the production of the acid by the wounded seeds after they have been cut was also considered. The time elapsing between cutting the seed and reading the pH is less than two minutes, and if the acid originates in this way one would expect it to continue to increase after this interval. Sometimes there was a slow increase of hydrogen-ion concentration in the solution, coming to a definite end-point from five to ten minutes after the indicator had been added. In these cases the change is probably accounted for by a diffusion of material from the uninjured cells of an embryo incompletely macerated. In other cases, the germ having been completely crushed, no such increase in acidity took place. Moreover, when the germs were removed as quickly as possible and macerated directly in a solution of the indicator, no indication of a gradual production of acid as a result of the wound could be seen.

The results obtained in this investigation may be summarized. Spinach seeds in their natural condition are surrounded by structures that decrease the rapidity of oxygen supply. As a result of this, the germination of all the vital seeds cannot be obtained at the temperatures ordinarily used in seed laboratories unless artificial arrangements are made to increase the amount of available oxygen. In samples of ordinary strength, complete germination is obtained at low temperatures. There are reasons for

believing that the explanation of the germination results lies in the fact that at higher temperatures the rapidity of reaction, combined with the slowness of oxygen supply, produces a toxic product as in fermentation. Spinach is thus found to be a plant that in nature would be handicapped by anatomical details in a struggle for survival in warm countries.

In conclusion, a word may be added on certain applications which the work has suggested. The relation between temperature and an active cell's requirements in the matter of rate of supply of raw materials may be found of importance in more ways than may have been realized, not only in germination problems, but as a factor in the ecology and geographical distribution of plants. There may in many cases be a relationship between anatomical structure and the temperature conditions required for the success of a plant. In green plants, in so far as oxygen is concerned, the problem is often largely overcome by the production of this gas as a by-product of photosynthesis. The question arises, however, whether inadequate facility of oxygen supply may not be sufficient to account for the comparative scarcity of large fleshy fungi in tropical forests and of large algae in tropical seas. The absence of heavy bud scales and thick bark from many rain-forest plants is doubtless of positive advantage from the standpoint of rapid oxygen supply, and the spreading of active chlorenchyma over stems and branches of cacti and other heat-enduring forms must be of value in providing oxygen to the adjacent tissues without the necessity for conduction from a distance.

SUMMARY.

Spinach produces starchy seeds with thin seed-coats, which remain enclosed in the fibrous ovary until germination. At ordinary room temperatures in ventilated chambers, a certain percentage of the viable seeds fail to germinate. In samples of ordinary strength, practically complete germination of vital seeds results from keeping the germination chamber packed in ice during the test. The same result is obtained by removing a part of the pericarp, by germinating uninjured fruits in an atmosphere of pure oxygen, or by subjecting them to a greatly reduced air-pressure for a short time twice daily during the germination period, thus producing a change of air within the pericarp.

During the progress of the test, a gradual rise in hydrogen-ion concentration, not due to carbon dioxide accumulation, takes place in the embryos of living but still ungerminated seeds. At the low temperature or with increased facility of oxygen supply, this concentration does not, before the end of the test, reach the point it has attained at the time germination stops in air at room temperature. It is believed that the failure of some seeds to germinate under the latter condition is due to the production of deleterious

organic compounds, as in fermentation or in muscular fatigue, the oxygen supply being too slow to complete the rapid oxidational changes taking place.

Other examples from the Plant Kingdom are cited, where structural characters may be instrumental in affecting the ability to withstand comparatively high temperatures.

BOTANICAL LABORATORIES,
UNIVERSITY OF TORONTO,
January 25, 1927.

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 2. KIDD, F.: Controlling Influence of Carbon Dioxide in the Maturation, Dormancy, and Germination of Seeds, Parts I and II. *Roy. Soc. Proc.*, vol. B. 87, 1914.
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EXPLANATION OF PLATE XXV.

Illustrating Mr. H. B. Sifton's paper on the Germination of the Seed of *Spinacia oleracea, L.*, at Low Temperatures.

Fruits and seeds of *Spinacia oleracea, L.*, photographed at a magnification of seven diameters.

Fig. 1. Complete fruit.

Fig. 2. Fruit with one half of pericarp removed, exposing the seed.

Fig. 3. Seed removed from pericarp.

Fig. 4. Fruit and seed bisected through greatest diameters.



1.



2.



3.



4.

SIFTON-SEED OF SPINACIA.

Huth coll

On Carpel Polymorphism. II.

BY

EDITH R. SAUNDERS,

Late Fellow of Newnham College, Cambridge.

With two hundred and twenty-seven Figures in the Text.

A RECENT reinvestigation,¹ in the light of the theory of the leaf-skin, into the nature of epigyny in certain typical cases involved as a consequence a fresh examination of the perigynous condition.² For this latter study the choice naturally fell upon the Rosaceae. This work at once brought to light the polymorphic character of the carpels in several pentamerous forms selected for examination (species of *Pyrus* and *Cydonia*), a result which in turn called for a wider survey of this family from this point of view. The conclusion that in those Pomoideae having a fully developed gynoeceium there are ten (not five) carpels, those of the outer whorl being sterile, those of the inner fertile, is in accord with the relations which the earlier contributions on this subject have already shown to be of very general occurrence.³ The reduced number in allied forms having an oligomerous gynoeceium, such e.g. as *Pyrus Aucuparia*, Gaertn., and species of *Crataegus*, is obviously due merely to suppression, owing probably to want of space, of one or more members in each carpellary whorl. But it remained to discover the nature of the carpel relations where reduction has been carried to the limit, as in some species of *Alchemilla*, *Acaena*, *Cliffortia*, *Poterium*, in *Margyricarpus*, in *Prunus*, and in the Cercocarpeae. In view of the outcome of the observations on the Pomoideae the evidence which had hitherto been accepted without question as indicating G 1 in the genera above mentioned clearly demanded re-examination. There were also various exceptional features in certain isomerous types which required explanation, as e.g. the varying position of the (supposedly valve) carpels in pentamerous Spiraeaceae, and the more or less bilocular character of the

¹ The Inferior Ovary. New Phytologist, vol. xxiv, p. 179, 1925.

² Perigyny and Carpel Polymorphism in some Rosaceae. Ibid., vol. xxiv, p. 206, 1925. (For the Leaf-skin Theory of the Stem, see Ann. Bot., vol. xxxvi, 1922.)

³ Loc. cit., and Carpel Polymorphism. I. Ibid., vol. xxxix, pp. 123-67, 1925; also vol. xxxvii, p. 451, 1923.

ovaries in *Amelanchier*. By extending the investigation to all sections of the family it was hoped to clear up these and other points.

Besides these inquiries touching the Rosaceae, certain further observations have been carried out on the Papaveraceae, Cruciferae, Begoniaceae, Droseraceae, Liliaceae, and other families which have been dealt with in detail or cited in the earlier accounts.¹ The Eucryphiaceae, Violaceae, and Passifloraceae, not previously discussed, are also briefly considered.

ROSACEAE.

Sanguisorbeae (Figs. 1–25 and 195, 196).

The *Sanguisorbeae* are described as commonly having the gynoeceum reduced to one or two *1-carpelled* ovaries, though in two of the genera investigated, viz. *Poterium* and *Alchemilla*, as many as four are found in some species. As will however appear from the facts set forth below the ovary in this group is in reality *dimerous*, consisting of one fertile and one sterile carpel.

Poterium. The ovaries in those species having two or more are separate for the greater part of their length but coalesce at their base. They are enclosed with the terminal style filaments in the cup of the four-partite perianth from which protrude at the top the stigmatic brushes. Five species were examined, viz. *P. canadense*, A. Gray, and *P. obtusum*, Franch., and Sav., with a single ovary or rarely two; *P. Sanguisorba*, L., and *P. verrucosum*, Ehrenb., ovaries generally two; *P. spinosum*, L., ovaries three or four. A transverse section of the top of the flower stalk shows four vascular bundles surrounding a small amount of pith (Figs. 1, 7, 15). From these four bundles arise the four cords which, running at first horizontally outwards and then upwards, furnish the midribs of the four perianth segments (Figs. 8, 11, 16). The residual vascular tissue remaining in the centre is used up in the formation of the ovaries. Each ovary is supplied with two separate vascular strands. One, the finer of the two, on the old supposition that the ovary is made of one carpel, would represent the midrib; the other, less attenuated, and regarded hitherto as corresponding to the conjoined edges, separates some way up the ovary wall into two distinct bundles. From one of these bundles a branch runs to supply the solitary ovule, the other being sterile (Fig. 25). Both these bundles, as well as the midrib, are continuous up the whole length of the style filament and jointly supply the bifid stigma (Fig. 25). *The origin and position of these fertile and sterile strands furnish evidence that each ovary is composed of two carpels.* After the emergence of the perianth cords the residual vascular tissue usually becomes concentrated in two masses as shown for *P. verrucosum* (Fig. 8),

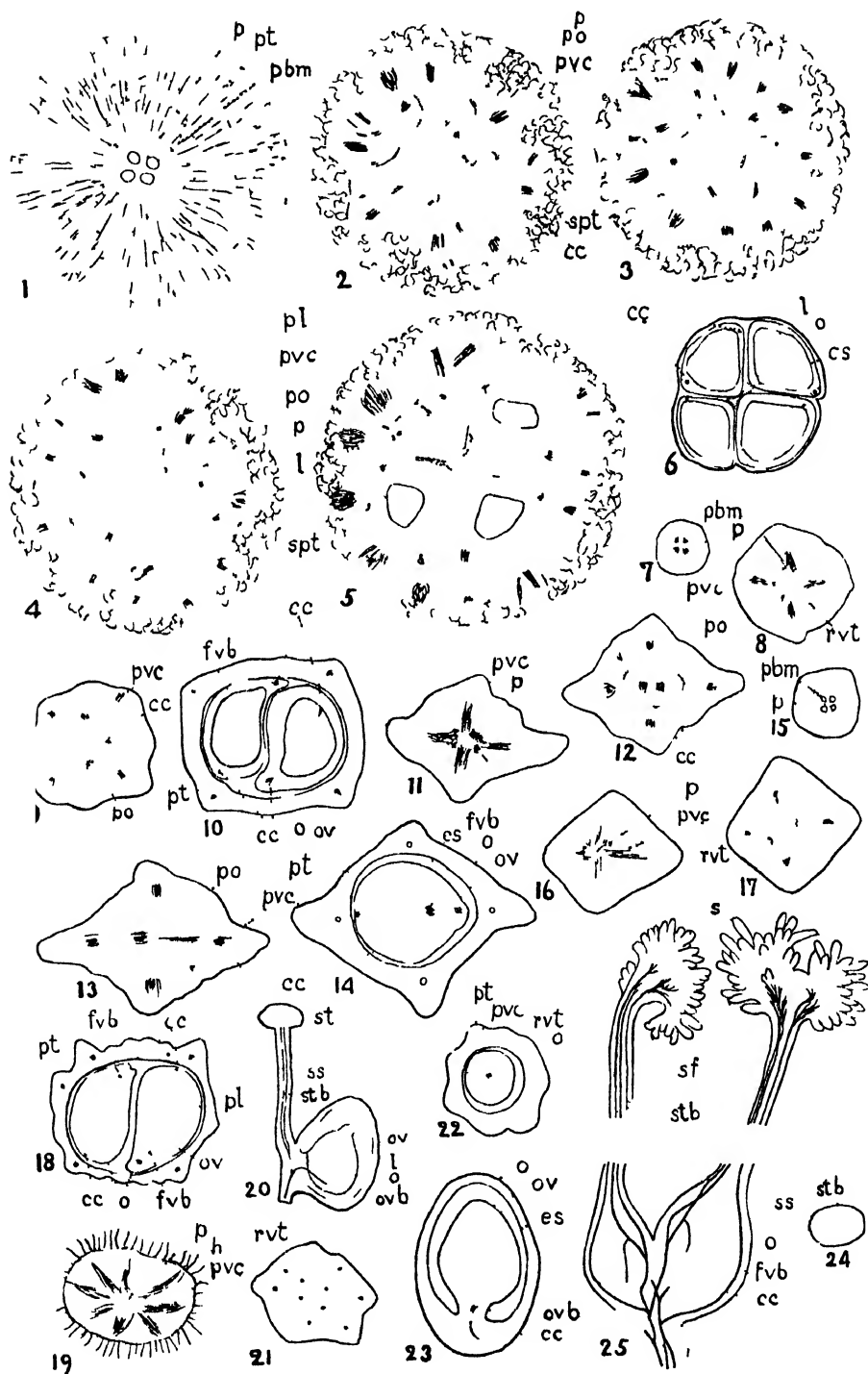
¹ Loc. cit.

P. canadense (Fig. 12), and *P. Sanguisorba* (Fig. 17). Differentiation of the gynoecium may follow directly, in which case only a single ovary develops as in most flowers of *P. canadense* and *P. obtusum*, the two masses passing entire to opposite sides of the ovary wall to form the cords of the sterile and the fertile member respectively (Fig. 13). But occasionally in the former species, and commonly in *P. verrucosum* and *P. Sanguisorba*, two ovaries are formed. When this is the case the residual vascular masses do not differentiate immediately, but retain their axial and embryonic character, each forking to form two new cords. The two pairs of cords thus formed supply, as in the preceding case, the two ovaries (Fig. 9) which thus arise by a process of 'twinning' or bifurcation of the axial cylinder, a condition of fairly frequent occurrence in certain Rosaceous types characterized by great reduction in the gynoecium¹ (see the account of various species of *Prunus*, as well as other genera in this same section). In *P. spinosum* the three or four pairs of cords arise apparently in this same way² (Figs. 2-5); they are seen occupying four areas surrounded on all sides by ground tissue (cortex, rays, and pith), an arrangement altogether inexplicable on the current view that each ovary is monocarpellary.

The solitary ovary continues, as it develops, to retain its original rounded shape (Fig. 14), but when two or more are produced they become flattened and turned slightly askew owing to the unequal development of the two carpel types, and the increasing mutual pressure as growth proceeds. In the case of either two or three they become pear-shaped in cross-section, the broad end of each fitting against the narrow end of its next neighbour (Figs. 10, 18). If four are formed the outline of each becomes triangular, with an angle instead of a side (as in the preceding case) directed towards the main axis (Fig. 6). It is to be noted that whether the number of ovaries be 2, 3, or 4 the corresponding 4, 6, or 8 vascular strands occupy positions on a circle, the ovaries standing tangentially to the main axis, a disposition difficult to reconcile with the orthodox view. Another fact at variance with the current belief is the absence, in the flowers of *P. spinosum* with four ovaries, of that regular alternation of fertile and sterile vascular strands as we travel round the circle, which we should expect to find if each fertile cord really represented the conjoined edges of a single carpel, of which the neighbouring sterile bundle formed the midrib. Instead, the four ovaries were found to be turned towards one another in varying ways. Here two fertile strands would stand next each other, there two sterile

¹ Certain Leguminous types in which it is not uncommon to find extra pods show the same phenomenon. In specimens of *Baptisia alba*, for which I am indebted to Mr. W. J. Dowson, this condition was observed to occur in quite a number of the lower flowers.

² The alternative solutions in this case of either a single whorl of eight carpels or of two tetramorous whorls condensed into one arising from a common apical point, neither of which is altogether ruled out, seem less probable than the one suggested, in view of the irregular distribution of the fertile and sterile cords mentioned later.



FIGS. 1-25. *Sanguisorbeae*. [All from transverse sections taken at successively higher levels except 20 and 25.] 1-6. *Poterium spinosum*, L. (ovaries 4). 1. Perianth tube base and vascular cylinder. 2-4. Successive stages in development of the numerous vascular bundles destined for the four perianth segments and of the boundaries of the four ovaries which are not yet separated from the surrounding tissue. Two vascular bundles, one strong and one weak, representing a fertile and a sterile carpel respectively, are formed in each ovary area, the whole number (8) occupying points on a circle. 5. Delimitation of the four ovaries from the pith and from each other is in progress towards the centre, but as yet there is no actual separation of the tissues. 6. The pith has disappeared, leaving a central space; the ovaries are now distinct in the centre, but are still united on their flanks. 7-10. *P. verrucosum*, Ehrenb. (ovaries 2). 7. The flower stalk. 8. The flower base at the level at which the four bundles for the perianth segments turn outwards leaving two residual vascular masses in the centre. 9. The limits of the ovaries are indicated, but not yet strictly defined; each has a stronger vascular bundle and a weaker one containing only a single xylem vessel. 10. One ovary is completely and the other almost completely free from the enveloping perianth tube; the broad end of the one fits against the narrow end of the other. 11-14. *P. canadense*, A. Gray (ovary usually 1). 11. The flower base at the level at which the vascular bundles for the perianth segments turn outwards. 12. The boundary of the ovary is now indicated within which are the two carpel cords. 13. The two vascular bundles leave the centre and pass entire to opposite sides of the ovary wall. 14. The single ovary with a solitary ovule. 15-18. *P. Sanguisorba*, L. (ovaries 2). 15. The flower stalk. 16. The flower base; the four bundles for the perianth segments have turned outwards, leaving a small amount of residual vascular tissue. 17. This residual tissue has become condensed into two opposite masses. 18. The two ovaries; each is composed of two carpels (indicated by the two vascular strands) and contains a single ovule. In the ovary on the left the fertile cord has divided in two and a furrow in the outline corresponds with this split. The appearance of two separate vascular strands in the funicle of one ovule is due to the plane of section having cut the ovule bundle twice—on its earlier upward, and again on its later downward course (see also Fig. 25). 19-24. *Alchemilla fissa*, Schum. (ovary 1). 19. The flower base; the eight bundles passing out from the centre furnish the midribs of the calyx and corolla. 20. The whole gynoeceium, showing the single ovary, lateral style, and solitary ovule; the vascular bundle for the ovule takes first an upward and then a downward course. 21. The residual vascular tissue after the passage outwards of the perianth cords is composed of four fine strands. 22. The four strands have condensed to form the midrib of the fertile carpel of the ovary, which is not yet wholly separate from the perianth. 23. The ovary below the level of origin of the style, with ovule. 24. The style with twin vascular strands forming a continuation of the fertile carpel bundle. 25. *Poterium canadense*, A. Gray. An exceptional gynoeceium of two ovaries rendered transparent and mounted whole. The origin and course of the funicle bundle can be traced; also the course up the style filament and into the stigmatic brush of the twin bundles of the fertile carpel and the single bundle of the sterile carpel.

The following abbreviations have been used throughout in explanation of the figures: *a*, anther; *acb*, anterior carpel bundle; *b*, beak; *br*, bract; *cb*, compound bundle; *cc*, carpel cord; *cs*, central space; *ct*, conducting tissue; *dc, dc'*, dorsal cords; *dvt*, discarded vascular tissue; *e*, endocarp; *es*, embryo sac; *f*, furrow; *fb*, flower bud; *fc*, fertile carpel; *fcb*, fertile carpel bundle; *fvb*, funicle vascular bundle; *g*, gynoeceium; *h*, hairs; *i*, integument; *ib*, integument bundles; *ivr*, inner vascular ring; *l*, loculus; *lb*, lateral bundle; *lf, lf'*, leaf bundles; *mt*, mechanical tissue; *o*, ovary; *ov*, ovule; *ovb*, ovule bundle; *ovr*, outer vascular ring; *p*, pith; *pa*, position of axis boundary; *pb*, petal bundle; *pbm*, primary bundle masses; *pc*, perianth cords; *pe*, petal; *pl*, position of loculus boundary; *plb*, placental bundle; *po*, position of ovary boundary; *ps*, papillose cells of the stigma; *pt*, perianth tube; *pvc*, perianth vascular cords; *r*, radius; *rvt*, residual vascular tissue; *s*, space; *sb*, sepal bundle; *sc*, stylar canal; *scb*, sterile carpel bundle; *se*, sepal; *sf*, style filament; *spt*, spongy tissue; *ss*, single style; *st*, stigma; *stb*, style bundles; *vb*, vascular bundle; *vc*, vascular cylinder; *vt*, vascular tissue; *wb*, wing bundle; *xy*, xylem bundle.

strands would be neighbours, while the remaining four would be alternately fertile and sterile (Figs. 3-6). Neither of these facts, on the other hand, is out of harmony with the present interpretation which regards the *Poterium* ovary as dimerous, a view which, even if no other evidence were forthcoming, would seem to be established by the fact of the utilization in the development of the single ovary (in species where only one is present) of two separate vascular cords *situated on opposite sides of the centre*. Were such an ovary in truth monocarpellary it would be necessary to admit that this solitary carpel terminates the stem. That a leaf structure can be terminal has recently been demonstrated by A. Arber¹ for certain Bamboos in which the imperfect spikelet is reduced simply to the flowering glume. But this condition is undoubtedly of very rare occurrence, and possibly may only be found in the last stage of reduction preceding complete disappearance of the foliar member. It can hardly be accepted as a satisfactory explanation in the case of a functional gynoeceum, especially when it reaches the degree of development found in such a type as *Prunus*, which presents the same problem. On the other hand, when the dimerous character of the *Poterium* ovary is once recognized we find less difficulty in understanding what has come to pass in such types as *Alchemilla* and *Agrimonia*, where the process of reduction has been carried even farther. Indeed, such a species as *P. verrucosam* is but one degree removed from the condition obtaining in these two genera, the xylem of the vascular cord of the sterile carpel often showing but a single vessel.

Acaena, *Cliffortia*, *Margyricarpus*, *Spenceria*. In *Acaena ovalifolia*, Ruiz. and Pav., *Cliffortia polygonifolia*, L., and *Margyricarpus setorus*, Ruiz. and Pav., the solitary ovary was found to be similar in structure to that of *Poterium*, but in *Acaena* the somewhat larger ground-plan and more fully developed vascular cords enable one to follow with greater ease the development of the two large residual vascular masses into the two carpel cords. In the specimen examined the two good-sized masses occurred on either side of one of the perianth cords; that is to say, they were situated on adjacent radii instead of on opposite sides of the axis centre as in *Poterium*, though it may be that this relation is subject to variation. In view of the tendency in many of the Sanguisorbeae for the sterile carpel cord to become extremely attenuated it is of some interest that in *Acaena ovalifolia* the two cords should be so equal in size that at the level of their origin it is not possible to decide with certainty which will be fertile and which sterile (Fig. 195). In *Spenceria ramalana*, Trim., where two ovaries generally develop and where bipartition of the central cylinder precedes differentiation of the carpel cords, the large amount of residual vascular tissue makes it particularly easy to follow the steps in this process. As the two halves

¹ Studies in the Gramineae. I. The Flowers of certain Bambuseae. Ann. Bot., vol. xl, p. 467, 1926.

draw apart a certain amount of phloem is left in the centre between them and is discarded (Fig. 196). Notwithstanding the much larger size of the ovary here the sterile carpel cord, as in *Poterium*, shows a minimum of xylem.

Alchemilla. This genus, like *Poterium*, includes some species with four separate ovaries (*A. nivalis*, H. B. and K.), some with three (*A. rupestris*, H. B. and K.), several with two, and others again with only one, as the British species *A. arvensis*, Scop., *A. vulgaris*, L., *A. alpina*, L., its derivative (?) *A. conjuncta*, Bab., and *A. fissa*, Schum., among others. The two latter forms were used for the present investigation. A cross-section through the top of the flower stalk shows a small central stele with little or no pith. From this central column there emerge almost simultaneously eight bundles which, passing outwards and upwards, supply the sepals (4) and petals (4) (Fig. 19). The residual xylem available for the gynoecium¹ is reduced to four very fine strands, each consisting of only one or two vessels (Fig. 21). These xylem groups are apparently insufficient to supply more than a single carpel, for they soon converge to a point on one side, merge (Fig. 22), and pass up into the solitary ovary as a single cord which pursues an upward course, giving off on its way a branch for the ovule (Figs. 20, 23). The style, which, unlike that of *Poterium*, originates near the base of the ovary on the ventral (placental) side, shows two fine xylem strands (Figs. 20, 24) which run separately up to the capitate stigma. These two strands form a continuation of the vascular cord which entered the ovary from below and furnished the bundle supplying the ovule. But here the reduction process has gone so far that, although the procambial tissue can be traced continuously from placenta to style base, there is for a short distance in this interval an actual discontinuity of differentiation and lignification. This fact may possibly account for Juel's statement,² which is incorrect, that the style of *Alchemilla* is destitute of vascular tissue. For a transverse section taken immediately above the level at which the bundle for the ovule curves out and downwards would show no xylem, while in one taken through the style the single minute vessel in each of the two strands might easily be overlooked. In the style rendered transparent and mounted whole they are quite easily seen. Along the dorsal line of the ovary, on the other hand, vascular tissue is wholly wanting. That is to say, where, on the orthodox view that the *Alchemilla* ovary is composed of a single valve carpel, there should be a midrib, there is none (Fig. 23). On this view the (presumed) marginal veins of the carpellary leaf must be supposed to arise otherwise than from a midrib and, in its absence, to be prolonged to supply

¹ The bundles for the four stamens have already passed out, conjoined with the petal cords, and become independent at a higher level.

² Die Blütenanatomie und Systematik der Rosaceen. Kungl. Svensk. Vet. Akad. Handl., lviii, p. 41, 1918.

the style and stigma—an unusual construction, to say the least, and one involving in such a case as *A. fissa* (where the ovary is solitary and the residual vascular tissue, consisting of four distinct and equidistant strands, converges to form the single vascular cord) the conception of a terminal carpel. The improbability of such a morphological relation, together with the fact that in no other section of the Rosaceae is a solitary ovary derived from a terminal carpellary leaf, furnish strong arguments against the single valve carpel interpretation: It seems, then, more logical to look upon the *Alchemilla* ovary as bicarpellary (as it undoubtedly will have been in some ancestral form), one member having, however, through reduction become entirely non-vascular.

Agrimonia. Suppression of the dorsal (sterile carpel) cord occurs in *Agrimonia* as in *Alchemilla*, although the two ovaries (three in some of the lower flowers) are here much larger, as is also the amount of residual vascular tissue. In the rearrangement which follows emergence of the perianth cords some scattered phloem strands lying peripherically never become reconstituted into definite cords, but run an irregular oblique course and shortly disappear. Here possibly we have the last remnants of the vascular tissue of the sterile carpel which in *Alchemilla* has vanished completely. The remaining xylem-containing masses become concentrated into two (or three) pairs of bundles, each pair forming the twin strands of the fertile cord of an ovary and passing eventually into the nearly terminal style. In the course of development the dorsal side of one of the two (or three) ovaries first becomes defined and gradually disjoined from the enveloping flower tube. As the process of separation continues the whole, now detached, mass of gynoeceum tissue becomes bisected and so gives rise to two free pistils. If three ovaries develop, as was the case in some specimens of *A. rupens*, L., this partition may take place in such a way as to produce a smaller and a larger half. The smaller portion develops directly into a single ovary, the larger repeats the cleavage process, each resulting half then giving rise to a whole free pistil. The reason, no doubt, for the successive division in the case of tripartition is that the three radii on which the ovary primordia are laid down are not equidistant as is inevitable in a flower with a pentamerous ground plan. It results that the two with least room for development remain continuous, with the contact boundary undefined, for a short distance after the third one has become completely free. As in the case of *Alchemilla* we may presume that part of the ovary wall is contributed by a sterile carpel from which all vascular tissue has now disappeared.

Prunoideae (Figs. 26–139).

Prunus. The familiar drupes of the genus *Prunus* (Cherry, Plum, Apricot, Peach, Almond), like the pods of the Leguminosae, have hitherto been regarded as typical monocarpellary fruits; the latter dry and dehiscent,

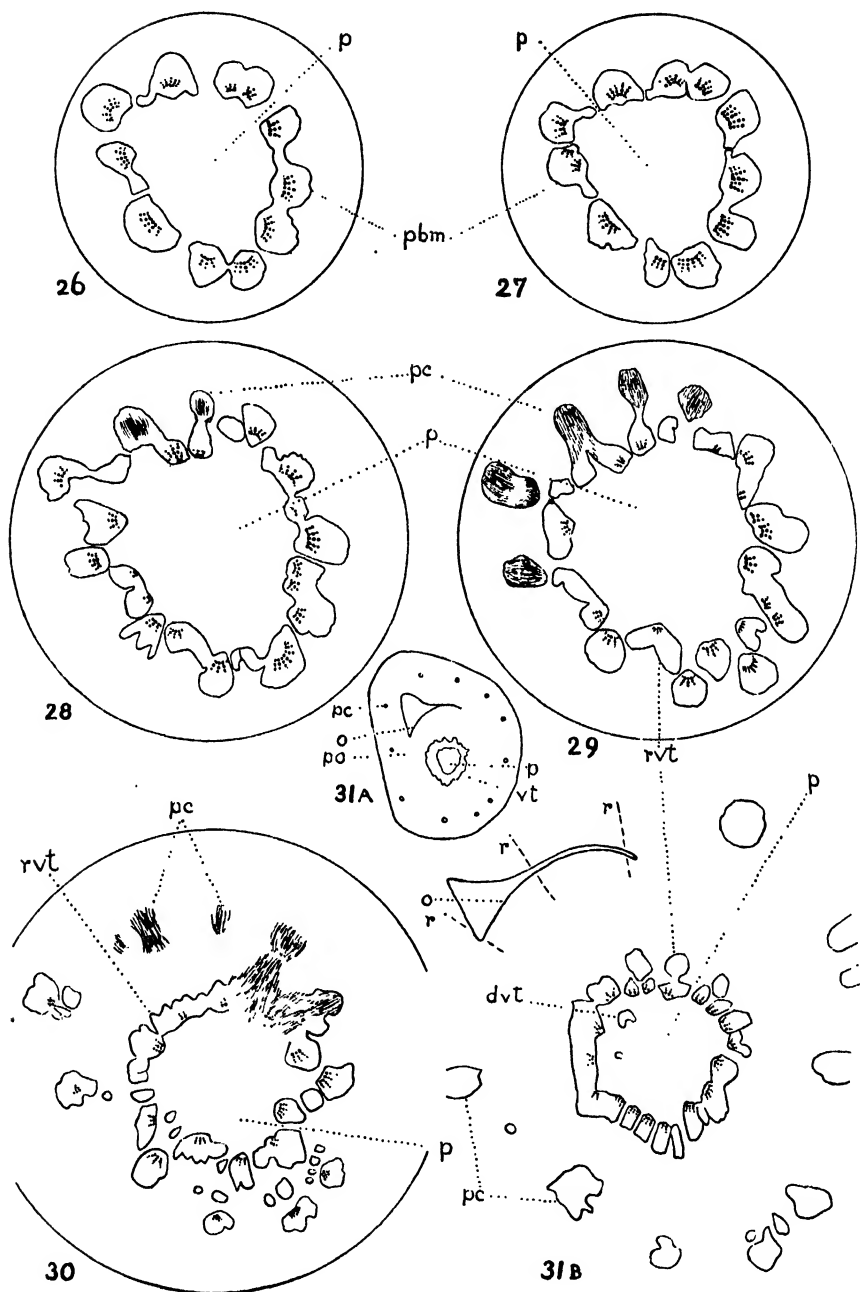
the former succulent or leathery and indehiscent. As will appear, however, from the evidence and arguments set forth below, the drupes of *Prunus*, like the legumes of the Papilionatae, are in reality dimerous. The leguminous ovary has already been discussed elsewhere,¹ the prunoid drupe we have now to examine more fully.

Although the conception of the *Prunus* gynoecium as an ovary composed of a single (valve) carpel has been universally accepted, investigation reveals numerous morphological relations which are not readily reconciled with this interpretation. In a flower with a 2-whorled pentamerous perianth and perigynous androecium, and a gynoecium in which one carpel out of an original whorl, also presumably pentamerous, alone survives, it is conceivable that this solitary carpel might sometimes be one particular member, sometimes another, and that the variable position of the ovary might thus be explained. But at least we should expect this surviving carpel to be so orientated that its midrib is backed against one of the perianth members, and its ventral suture directed inwards towards the axis line. But this is not the observed disposition. It would indeed seem that, on the strength of the suture-like appearance on the one side of the fruits, and the presence of a strong vascular cord on the opposite side, too ready an acceptance has been accorded to the 1-carpel interpretation, and that sufficient attention has not been paid to the internal structure. It will therefore be well, before discussing further the difficulties involved in the G 1 formula, to describe in some detail the manner in which the ovary is formed in those members of the genus which have been investigated.

Prunus Cerasus, L., Cherry (Figs. 26-48). Transverse sections taken through the top of the flower stalk show a ring of (usually) ten-bundle masses (Figs. 26, 27), as was found in the Pomoideae.² Above this level these primary masses break up irregularly, some portions passing outwards and upwards to furnish the midribs of the sepals and petals (Figs. 28-30). The residual portions left in the centre close up again to form a more or less continuous ring, and at the same time the ovary gradually becomes delimited from the tissue enveloping it (Figs. 31 A and B), separation taking place, in the particular flowers examined, first along the length of one side, then at both dorsal and ventral surfaces, and finally along the remaining side. From the reconstituted vascular ring there now again pass outwards numerous vascular cords, generally unequal in size, and emerging at points in succession from the dorsal to the ventral face (Figs. 32-7). A considerable amount of vascular tissue, chiefly phloem, simultaneously wanders into the pith, gradually replacing the parenchyma, which becomes reduced to a few small islands of tissue (Figs. 36, 37). At a point about opposite the

¹ Carpel Polymorphism. I. Ann. Bot., vol. xxxix, 1925.

² See Perigyny and Carpel Polymorphism in some Rosaceae, New Phytologist, vol. xxiv, p. 206, 1925.

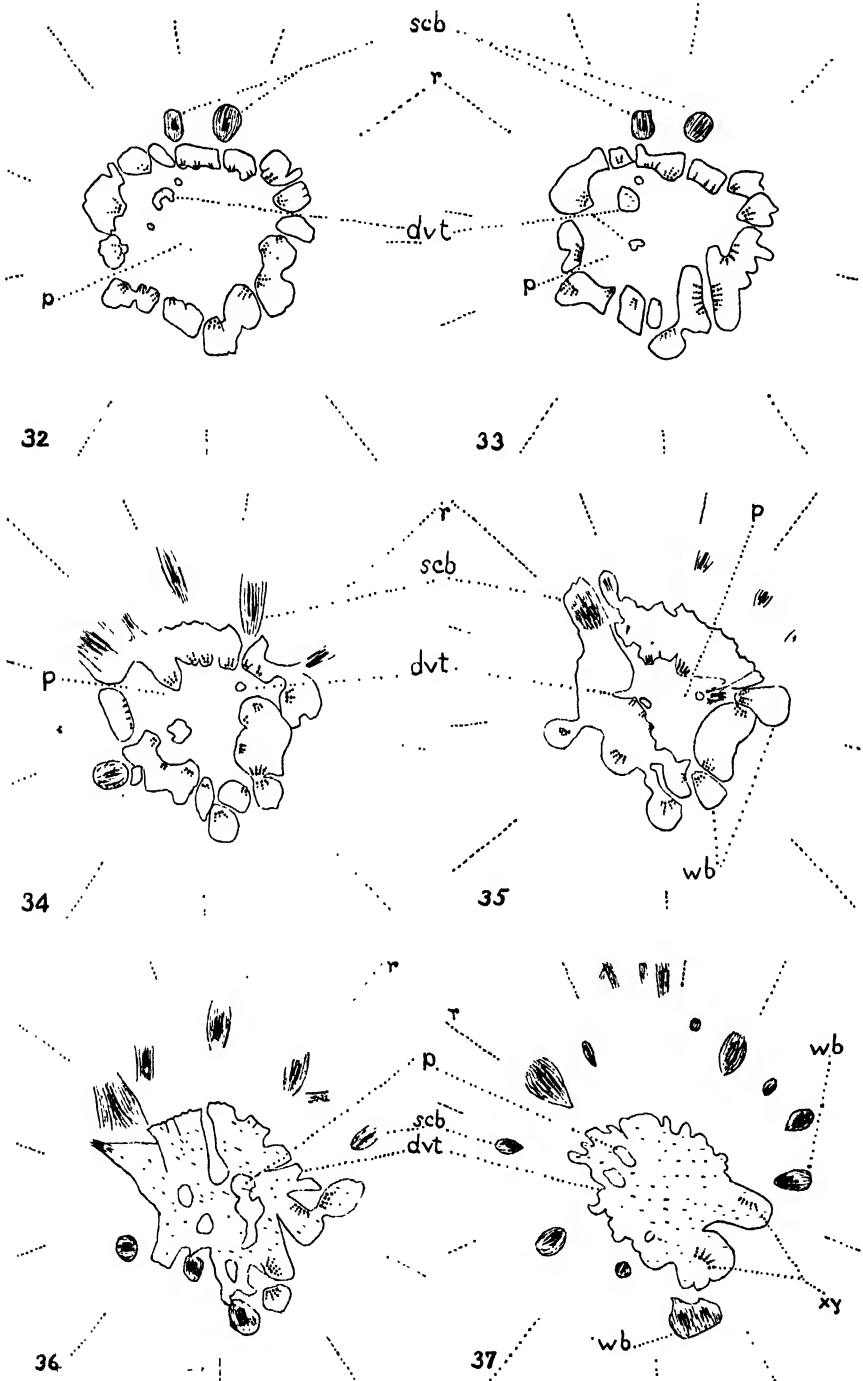


FIGS. 26-31. *Prunus Cerasus*, L. [All from transverse sections taken at successively higher levels through the same flower A.] 26. Flower stalk with ring of ten primary bundle masses. 27. Flower base; small portions branch off laterally from some of these bundle masses. 28. The primary masses are becoming more broken up. The vascular cords for the perianth segments can now be identified; two are seen cut obliquely as they leave the central ring. 29. More perianth

most prominent of these cords, the remaining xylem elements become concentrated into two groups, placed pairwise back to back, and these, with the adjacent phloem, constitute the characteristic double bundle of a single fertile cord (Figs. 37, 38). Above this level the amount of unused phloem rapidly diminishes and finally disappears, giving place again to a parenchymatous pith, which in turn comes to an end as the loculus makes its appearance (Fig. 39). At about this same level the twin bundles of the fertile cord each give off a branch which serves the corresponding placenta (Fig. 39). The successive stages in the development of these placental bundles are shown in greater detail in another specimen (Figs. 40 A-F). In *P. Amygdalus*, Stokes (Almond) (see Figs. 81-96), and *P. Armeniaca*, L. (Apricot), these stages can be traced perhaps even more clearly, since in these species the twin bundles of the fertile cord and the neighbouring sterile bundles appear to run a separate course throughout their length, whereas in the Cherry and other species some anastomosis usually occurs. It is in the oblique plane passing between these paired bundles of the fertile cord that the seam occurs which is noticeable on the surface of the Cherry fruit, and which has been supposed to indicate the line of fusion of the two margins of a single carpel (Figs. 39-42). But the evidence here, and in the cases which follow, all points to the conclusion that the seam tissue is not composed of the fertile *edges* of a folded valve carpel, but of a *whole* second fertile carpel interposed between the sterile margins of the other. The numerous sterile bundles present form the veins of the sterile member; the one situated opposite to that of the fertile carpel becomes more prominent than the rest, and forms the midrib (dorsal cord), while the four or five others on each side furnish secondary veins. Of these the one on each side of the fertile carpel is more strongly developed than the rest. Now these strong 'wing' bundles are *not* the ones which give rise to the placental strands, nor could they, were the ovary actually monocarpellary, be strictly described as marginal. In some species they are placed a considerable distance apart (see Figs. 35, 98, 137), the intervening space being occupied by a certain amount of phloem, some of which is later discarded, the rest being utilized to form the bundle of the second, non-expanded fertile carpel, which lies somewhat nearer the centre and is differentiated somewhat later. These two features make it probable that this second carpel belongs to an inner whorl, of which it is the sole surviving member.

As the ovary develops, a line of cleavage becomes apparent in the tissue beneath the seam, extending inwards from just below the surface in the direction of the placental cords (Figs. 39-41), at a higher level passing

cords leave the central ring. 30. The residual vascular tissue becomes re-formed as a ring. 31 A. The ovary begins to separate from the flower tube. 31 B. The central region of 31 A more highly magnified. A small quantity of discarded vascular tissue is seen in the pith. The direction of the perianth cords for those members now free from the ovary is shown by the broken lines *r*.



FIGS. 32-7. *Prunus Cerasus*, L. (continued). [All from transverse sections taken at successively higher levels through the same flower A. Only the central region is shown.] 32, 33. Two

between them, and finally becoming continuous with the loculus, here filled with conducting cell tissue (Figs. 42-4). This median radial splitting is, as we have seen elsewhere, a characteristic feature of consolidated carpels.¹

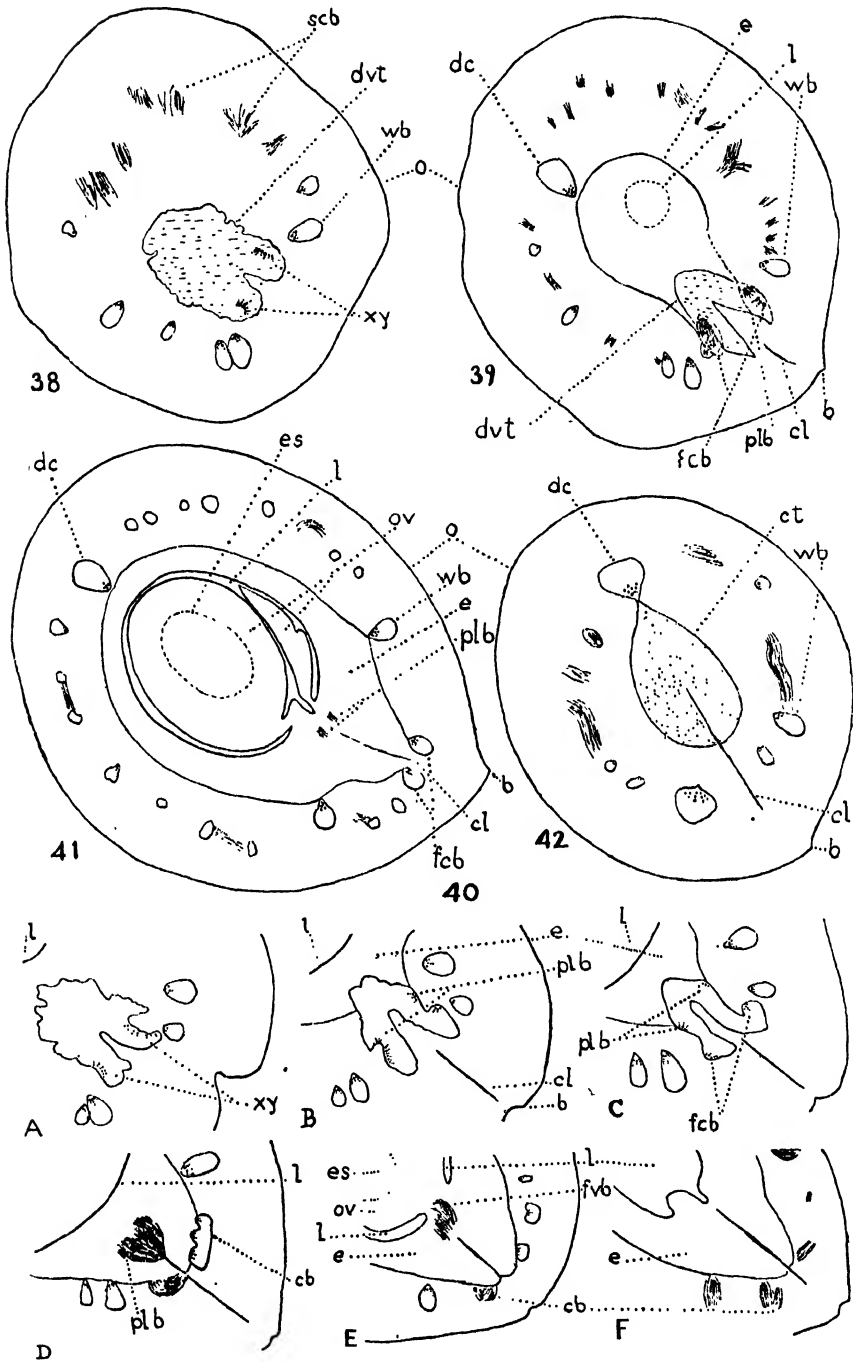
After supplying a vascular strand to the two funicles the fertile carpel ends come to an end (Fig. 43), the veins of the sterile carpel alone persisting above this level (Figs. 43, 44). As the base of the style is reached the smaller of these veins disappear or merge with the midrib and wing bundles (Fig. 45), which remain distinct and are traceable until the level of the stigma is approached, when they break up again into numerous smaller bundles (Figs. 46-8).

Thus we find that the semblance of a cherry to a unicarpellary type of fruit is illusory. That an identical appearance may be produced by one single large member, and by two or more smaller like members fused together, is, of course, quite well known. To find comparable cases we have only to look at simple (single) and compound (connate) styles, or at normal and fasciated cylindrical stems. Or, if we may venture to take an example from the animal kingdom, we may cite the case of 'mule-footed' swine. Here the undivided character of the hoof suggests an origin from a single toe, but examination of the skeleton reveals the fact that the foot is, in reality, 2-toed as in the normal animal. Similarly in the Cherry, the single seam suggests an origin from a single folded carpel, but the internal structure reveals the presence of a second member, and this we shall find holds good throughout the genus.

Prunus Laurocerasus, L., Cherry Laurel (Figs. 49-53). Transverse sections through the flower of this species show the same general ground plan as the Cherry, though they exhibit certain distinctive features. The origin and course of the ten perianth cords, five of which are stronger and alternate with the other weaker five, are particularly clear owing to the absence of branching (Fig. 49). After the passage outwards of these cords the central vascular ring re-forms. A little above this level the solitary ovary becomes free from the surrounding perianth tube, separation beginning (in the particular specimen investigated) along the ventral half of one side, extending from this region in both directions, the last portion to become severed being the dorsal half of the opposite side. In the re-formed vascular ring the xylem elements are concentrated into a strongly developed dorsal cord,

bundles destined for the sterile carpel have left the re-formed vascular ring. 34. More bundles are leaving the central ring to form the veins of the sterile carpel; one, already more prominent than the others, will become the midrib (dorsal cord). 35. The two bundles which will become the 'wing' bundles of the sterile carpel can now be distinguished. 36. A large quantity of vascular tissue, mainly phloem, has invaded the pith so that only small islands of parenchyma remain. 37. The residual xylem elements become concentrated to form the twin bundles of the fertile carpel (xy). The veins of the sterile carpel branch soon after they arise.

¹ See e. g. the account of *Lilium* and figures of Tulip and *Fritillaria* in Carpel Polymorphism. I.



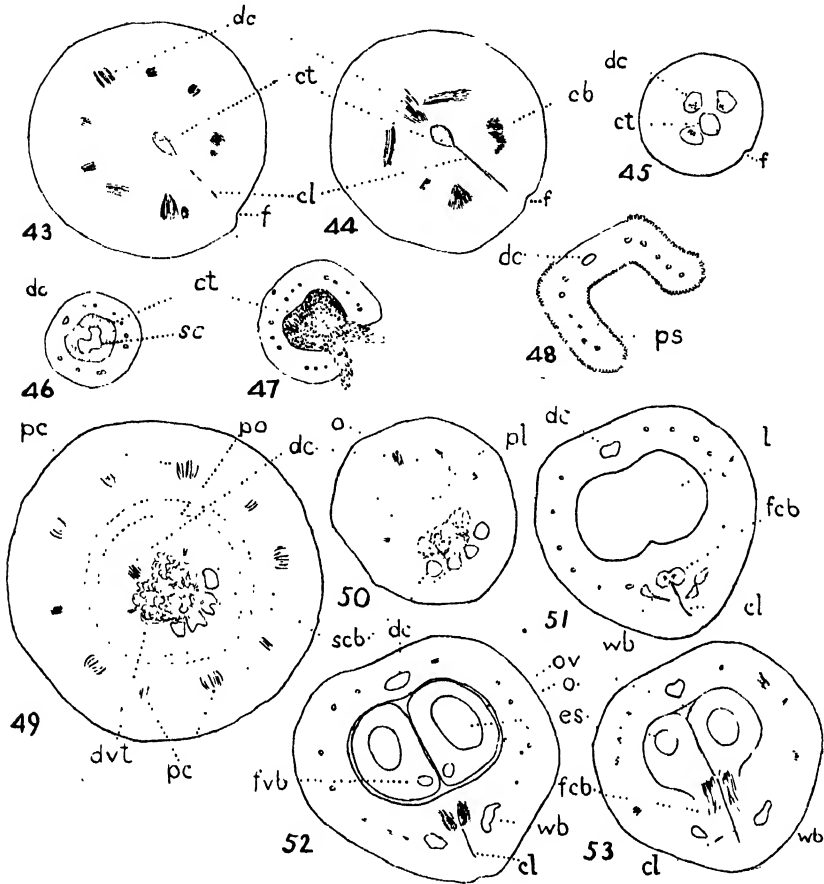
FIGS. 38-42. *Prunus Cerasus*, L. (continued). [All from transverse sections taken at successively higher levels; 40 from flower B, the rest from flower A.] 38. The sterile carpel veins branch as they turn outwards; the pith parenchyma has now entirely disappeared. 39. The midrib

one or two feebly developed bundles on each side of it, and opposite to it four well-developed bundles in close proximity to one another; the few remaining elements of the xylem, and a large amount of discarded phloem, are distributed through the pith (Figs. 49, 50). The vascular supply of the pistil is thus meagre as compared with the Cherry. At a slightly higher level the pith parenchyma finally vanishes, the loculus makes its appearance in front of the dorsal cord, and the last remnant of vascular tissue is concentrated on the opposite side of the loculus to form the twin bundles of the fertile carpel cord (Fig. 51). As the ovule-bearing region is reached a branch from each of these latter bundles runs to the corresponding ovule, the two being here equally developed (Figs. 52, 53). The appearance of the 'beak', cleavage line, and stylar canal, and the course of the vascular bundles in the style filament, resemble these same features in the Cherry, and call for no special comment.

Prunus lusitanica, L., Portugal Laurel (Figs. 54-63). This species was selected for comparison with the preceding forms because the flowers not infrequently produce two equal drupes, the origin of which, from a vascular arrangement like that of the Cherry, was not self-evident and demanded investigation. In the flower with a single pistil the course of events is very similar to that observed in *P. Cerasus*. We find at the top of the flower stalk the usual ring of primary bundle masses (Fig. 54), and at higher levels the emergence of the ten cords for the calyx and corolla (Figs. 55-7), and later, after re-formation of the vascular ring, the passing out of the veins of the sterile carpel, the invasion of the pith, and finally the concentration of the remaining vascular elements into the cord of the fertile carpel. In the specimen from which Fig. 54 was drawn, separation of the ovary from the flower tube occurred first along one side, then at the dorsal and ventral surfaces, and finally along the opposite side. When two drupes are produced, no foreshadowing of this duplication was to be seen in the flower stalk, unless a slight increase in the size of the bundle masses were to be regarded in this light. As the base of the flower is approached a strongly developed cord becomes distinct at each end of the now elliptically shaped vascular cylinder (Fig. 58). A strand on either side of each of these cords curves outwards and backwards from the ring, one sweeping round to the right, the other to the left in each case, the stronger of each pair taking

(dorsal cord) of the sterile carpel has become well developed; the twin bundles of the fertile carpel give off a branch to the corresponding placenta; the boundary of the endocarp and loculus can be traced. 40. Successive stages in the development of the placental bundles (A-C) and of the line of cleavage (B-F) in another flower. In D one of the bundles of the fertile carpel has fused with a wing bundle of the sterile carpel. 41. The placental bundles have moved inwards and are now at some distance from the main (midrib) bundles from which they arose. Each placenta bears an ovule; one becomes functional, the other aborts. The endocarp is well defined. 42. The loculus is closing in and is filled with conducting tissue; the placental bundles have come to an end and are no longer seen.

up a position more or less in the dorsal¹ median line, and becoming the



FIGS. 43-53. 43-8. *Prunus Cerasus*, L. (continued). [All from transverse sections taken at successively higher levels through flower C.] 43, 44. Apical region of the ovary. The dorsal cord, and the fertile carpel bundles, either separate or fused with the wing bundles of the sterile carpel, can still be traced. The line of cleavage has extended to the conducting tissue which fills the top of the loculus. 45. Basal region of the style filament with dorsal and wing bundles. The cleavage line has disappeared; a slight furrow marks the continuation of the seam (beak) of the ovary. 46, 47. Apical region of the style filament. 46. The dorsal and wing bundles have broken up into numerous smaller bundles. 47. The stylar canal has opened on to the ventral surface, the conducting cell tissue becoming continuous with the stigmatic papillae. 48. The stigma; stigmatic papillae are developed on both surfaces except behind the dorsal cord. 49-53. *P. Laurocerasus*, L. [All from transverse sections taken at successively higher levels, 49 and 50 from flower B, 51-3 from flower C.] 49. The ovary is becoming delimited, but is not yet free from the surrounding tissue. The dorsal cord, two weak secondary veins, and strong wing bundles are already differentiated; the pith is almost obliterated by discarded vascular tissue. 50. The ovary is now free, and the loculus is becoming defined. The discarded vascular tissue is diminishing. 51. The twin bundles of the fertile carpel are now differentiated, and the line of cleavage has made its appearance. 52, 53. A placental branch arises from each of the two bundles of the fertile carpel and runs to the corresponding ovule.

sterile carpel midrib. These diverging strands are followed by the remaining bundles of the ring in turn, until the original ellipse has become replaced

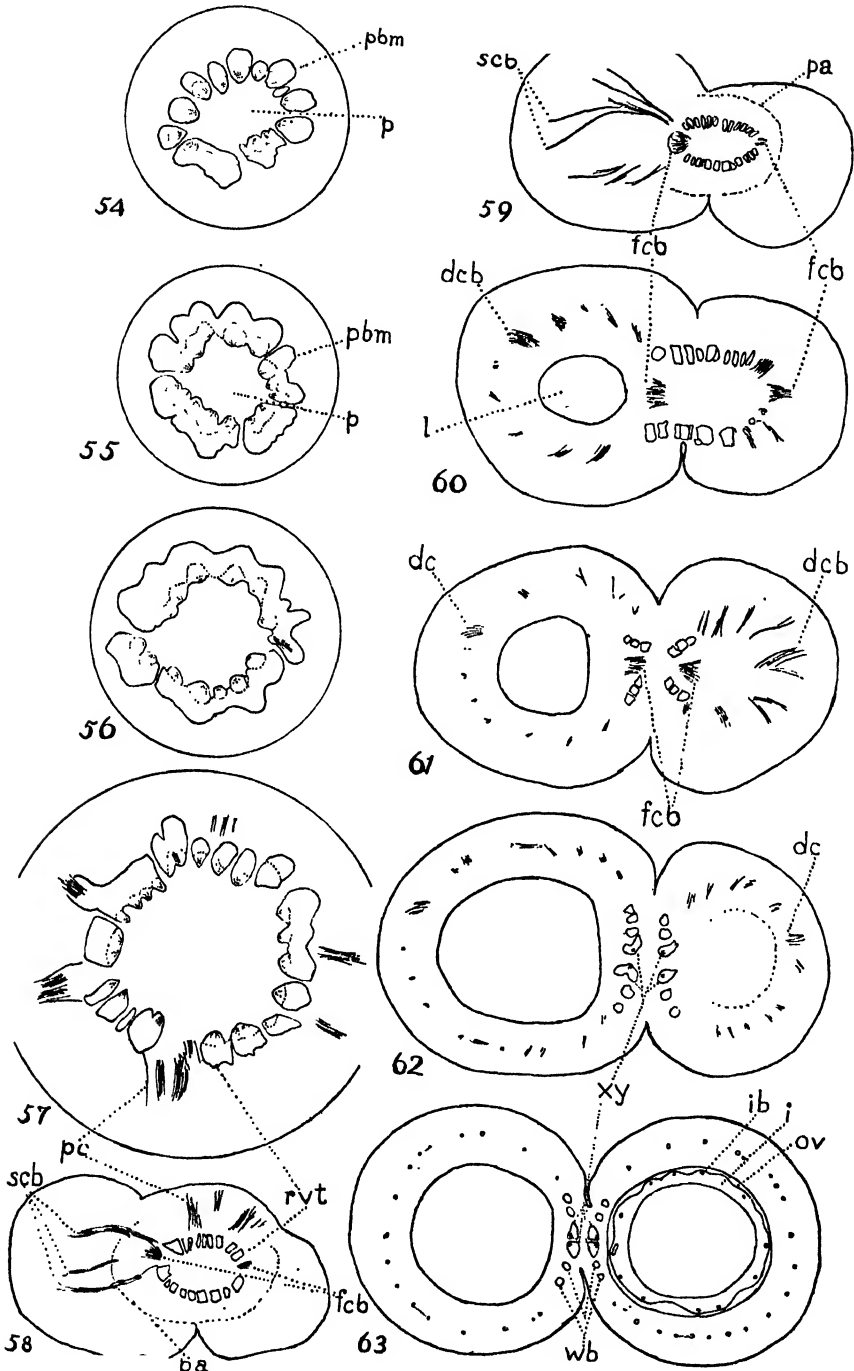
¹ Dorsal, i. e. as regards each drupe.

by two circles composed of the midribs and secondary veins of the two sterile carpels, except at the point where the circles approach one another. Here the bundle which was situated at each end of the long axis of the ellipse is now seen to face the other way, and furnishes the twin strands of the cord serving each fertile carpel, which thus occupies the position nearest to the axis centre, completing the circle on this side. This change of orientation is illustrated in the diagram on p. 587, and by Figs. 58-63, which are taken from successive sections through one flower.

Thus we find as a common though erratic feature in *P. lusitanica* that 'twinning' of the vascular system in the axis which occurs regularly in *Spenceria* and some species of *Poterium*. The residual vascular tissue in this species may then either be fashioned in its entirety into a single ovary or it may be bisected, a whole ovary being then developed from each half. The orientation of the fruit, both in the case of a single drupe and of a pair, is variable. If solitary, the seam visible on the exterior indicating the position of the fertile carpel is sometimes directed to the front of the flower, sometimes to the back, sometimes laterally. If two drupes are present they may stand in the median or the lateral plane, but whatever their position they are so orientated that the two seams face one another.

The formation of more than one pistil in a single flower is met with occasionally in other *Prunus* types. In a Plum tree examined in 1926 this peculiarity was observed in several of the early flowers, and the same condition is not uncommon in the first blossoms of the Almond. In a variety of Victoria Plum which frequently bloomed a second time in the season, for material of which I am indebted to Mr. F. T. Brooks, many of the flowers of the second period produced two, three, or even four ovaries.

Victoria Plum (Figs. 64-80). In the above-mentioned variety the arrangement of the vascular bundles of the flower stalk and the mode of origin of the perianth cords (shown in Figs. 64, 65, 72, 73) call for no special comment. In flowers in which twin ovaries are to be formed, the vascular ring becomes elliptical, and at the same time the whole gynoecium gradually separates from the surrounding flower wall (Figs. 66-9), sometimes in such a way that one ovary becomes free before the other, the dorsal surface being first to be disjoined in the one, and last in the other. Or severance may extend along both sides of both ovaries simultaneously, in which case the dorsal surface is the last to be exposed in both. In the former case (the one figured) the vascular bundles in one half of the ellipse move outwards into the protuberance formed by the earlier of the two ovaries to develop. The loculus of this ovary then makes its appearance, and a portion of the residual vascular tissue which has invaded the pith becomes concentrated on its inner (ventral) side, and gives rise to the twin bundles of a fertile carpel (Figs. 70, 71). This sequence is repeated in the development of the second ovary. (In the second type of disjunction mentioned above



FIGS. 54-63. *Prunus lusitanica*, L. [All from transverse sections taken at successively higher levels; 54 from a flower with a single ovary, the rest from another flower with two ovaries.] 54, 55. The flower stalk. 56. The flower base; portions of the vascular ring are being detached to

these various stages take place in both ovaries simultaneously instead of successively.) In this way the vascular tissue is divided about equally between the two, half the original ring of bundles passing into the one, and half into the other, while a median constriction in line with the short axis of the ellipse brings about separation of the one from the other. It will thus be apparent that the mode of utilizing the residual vascular tissue in the construction of two equivalent ovaries in the *Victoria Plum* differs from

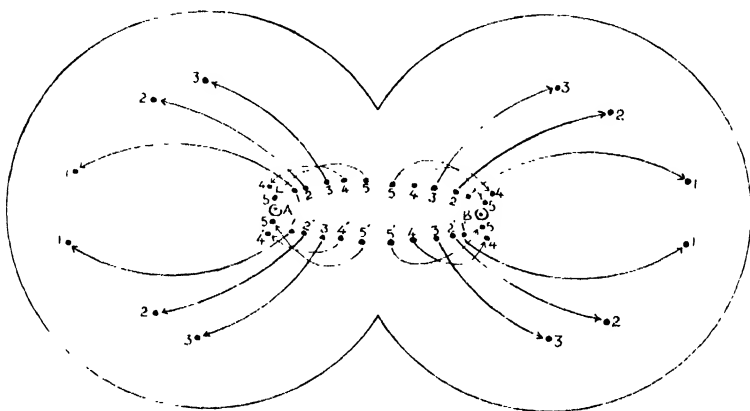
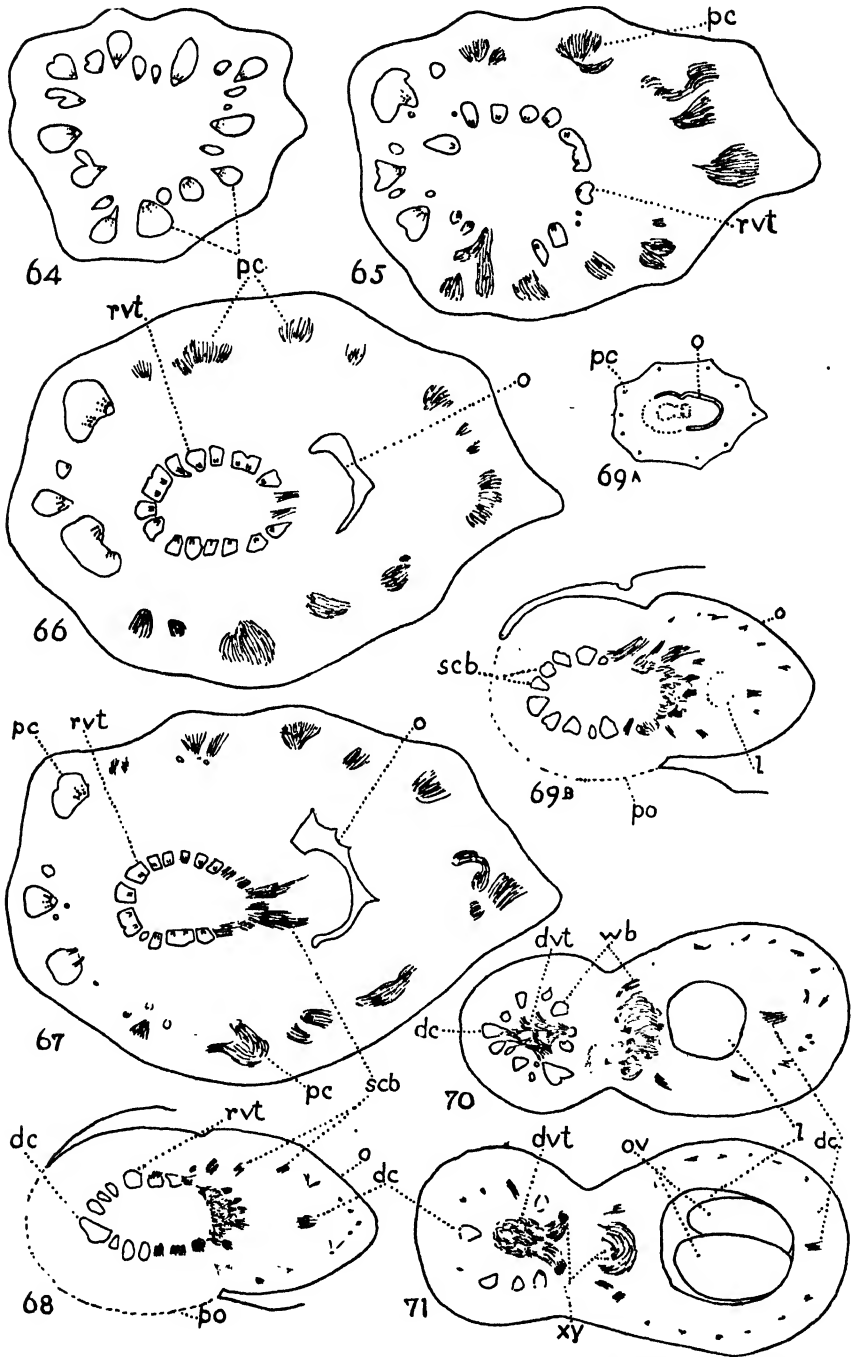


FIG. 63 A. Diagram to illustrate change of orientation of bundles in *Prunus lusitanica*.

that described above for the 2-drupe flower of *P. lusitanica*. Whereas in the latter case the bundle at either end of the long axis of the ellipse retains its position and furnishes a fertile carpel cord, the corresponding bundles in the *Victoria Plum* move outwards and form the more or less prominent dorsal sterile cord of each ovary. The position of the ovaries in regard to one another is, however, the same in the two types, the dorsal cord being always on the outer, the seam on the inner face when, as in the case described, the two have a similar origin. This is not always the case. In some of the flowers examined, in which more than one ovary was present, one alone would attain normal size, the others being small and incapable of functioning. In such cases the normal ovary was found to arise in the same manner as in the typical flower, the whole ring of vascular bundles being utilized in its construction without undergoing bisection (Figs. 74-8). The one or more extra ovaries were found not to spring from the stem apex, but from the inner face of the perianth tube, after the manner of a stamen (Fig. 79). Though similar in outline to the normally

form the perianth cords. 57. The perianth cords are emerging from the vascular ring. 58. Bundles from the re-formed vascular ring are passing out into the ovary on the left to form the midrib and secondary veins of the sterile carpel. 59. A later stage. 60, 61. These stages are repeated in the ovary on the right. 62, 63. The twin bundles of the fertile carpel are now differentiated in both ovaries; the integument of the ovule shows a ring of small bundles.

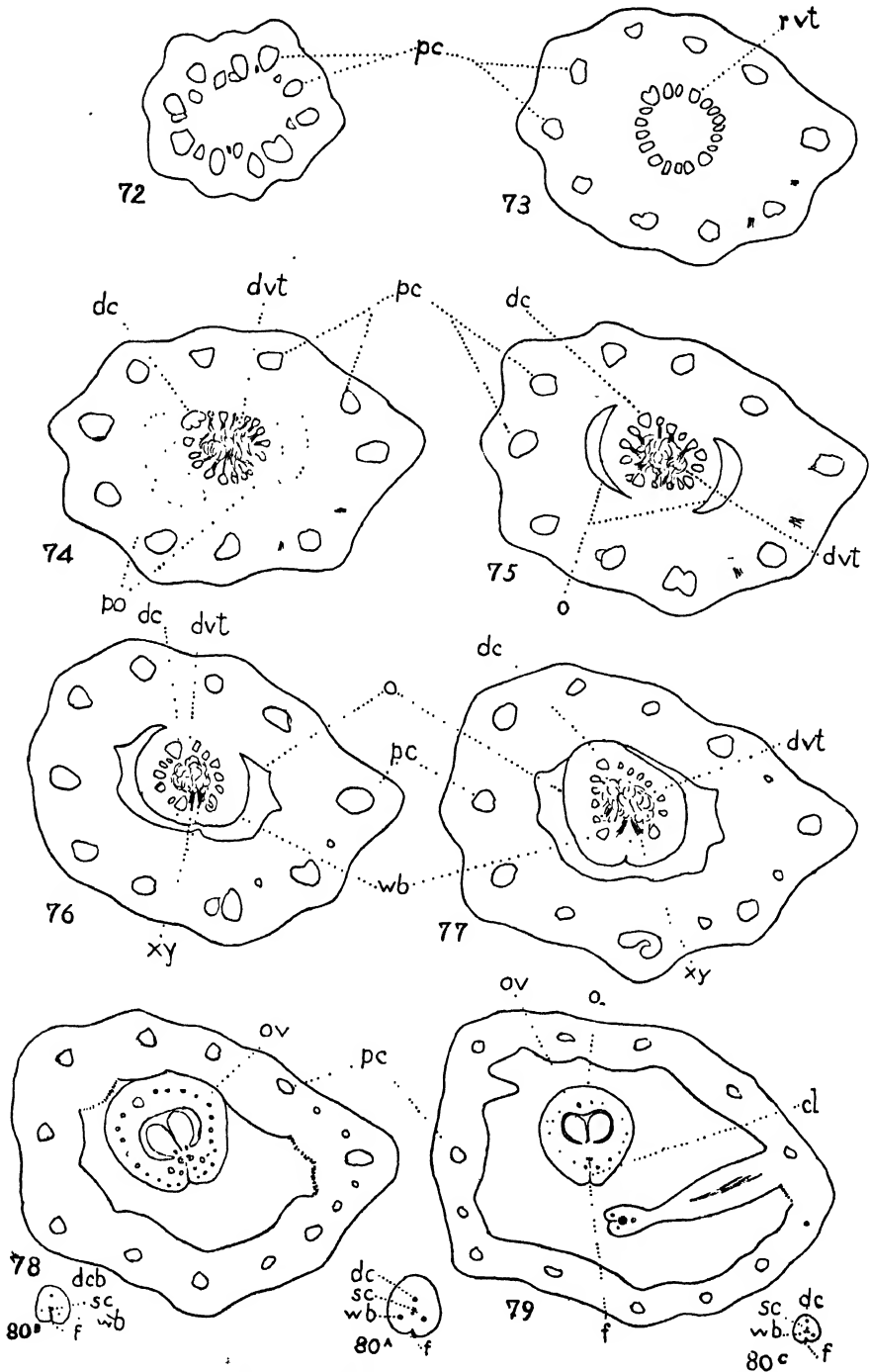


FIGS. 64-71. *Prunus*, sp. (Victoria Plum). [All from sections taken at successively higher levels through the same flower A with two equivalent ovaries.] 64. The flower stalk. 65. The perianth cords have left the vascular ring, which is re-forming. 66. The ovary on the right is becoming free from the flower wall. 67. Vascular bundles are leaving the central ring to enter the ovary

formed functional pistil, they are smaller, and do not develop either fertile cord or ovules. In a transverse section of the style region they show a central canal with the usual dorsal bundle and a wing bundle on either side of the ventral furrow (Figs. 80 A, B, C). Here we have an attempt to construct an ovary out of a single leaf member which takes on the form of the valve carpel. But the valve member being sterile, and no second carpel being present, the ovary is functionless. These supernumerary ovaries furnish further indirect evidence in proof of the dimerous nature of the normal organ. They are a common feature of the double-flowered forms of various species, but these will be dealt with in detail later (see p. 593).

Prunus Amygdalus, Stokes, Almond (Figs. 81-97). In this species, as in the Victoria Plum, it is not uncommon to find among the early flowers some with two approximately equal and functional ovaries, each with style filament and stigma. In normal flowers the arrangement of the vascular bundles of the flower stalk in a ring (Fig. 81), the origin and passage outwards of the perianth cords (Fig. 82), the delimitation of the ovary and the re-formation of a continuous vascular ring (Fig. 83), the entry into the pith of the superfluous vascular elements (Fig. 84), and the differentiation of the utilized portions to form the veins of the sterile valve and solid fertile carpels follow the ordinary course (Figs. 85-9). As the prominent dorsal cord turns outward from the vascular ring, followed in order of position by the smaller veins on either side, which in transverse section are thus seen cut obliquely (Fig. 84), the ovary gradually becomes free from the perianth envelope. In general, severance begins along the length of one side, but the succeeding steps follow no fixed rule, the last portion to become free being sometimes the opposite side, sometimes the ventral, sometimes the dorsal surface. Whichever may chance to be the mode of separation, on the old idea that $G = 1$, the single carpel must be conceived to be folded in a plane, not, as we should expect, perpendicular to the surface of the perianth tube, but parallel with it, a morphological relationship extremely difficult to accept. The cord of the fertile carpel shows the characteristic twin xylem bundles (Figs. 86-8), each of which gives off a fertile branch (Figs. 88, 89). In the Almond these bundles generally remain distinct from the strongly developed wing bundles of the sterile carpel. Shortly above the ovule-bearing region the fertile carpel bundles come to an end (Fig. 90), as also do the smaller veins of the sterile valve carpel, so that at the top of the ovary, and in the style, only the midrib (dorsal cord) and wing bundles of the latter member are to be seen (Fig. 91).

on the right. 68. These bundles have become differentiated into the midrib and secondary veins. 69 A. The right ovary is now wholly, and the left partially free from the flower wall. 69 B. The gynoecium more highly magnified. The bundles of the fertile carpel are in process of formation in the right ovary. 70, 71. Bifurcation of the vascular ring is now complete, and the ovaries are beginning to draw apart. The bundles of the fertile carpel are still in process of formation. The loculus has not yet made its appearance in the left ovary, the centre of which is occupied by discarded vascular tissue.



FIGS. 72-80. *Prunus* sp. (Victoria Plum) (continued). [All from transverse sections taken at successively higher levels from another flower B with one terminal and two supernumerary ovaries.]

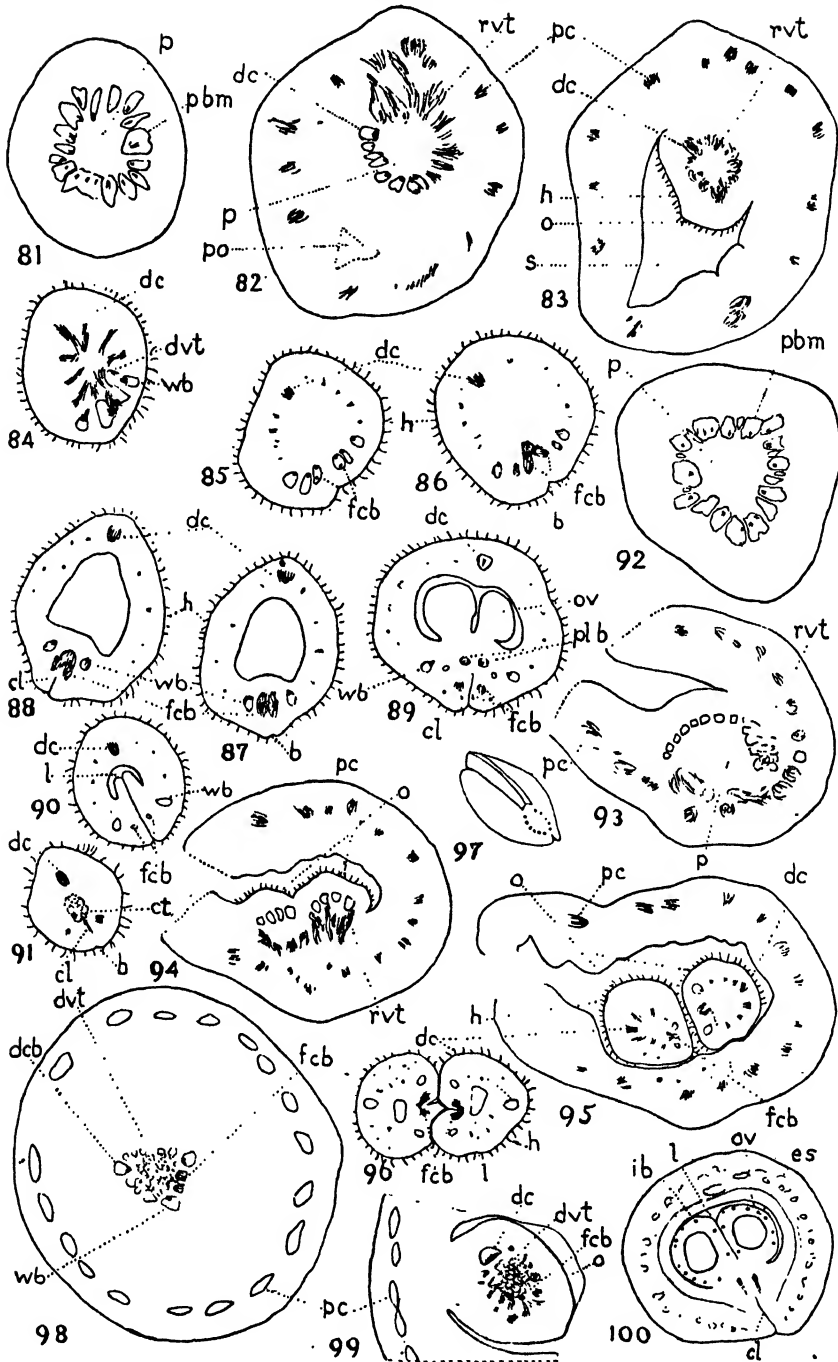
In a flower with two central ovaries a single protuberance first makes its appearance as the perianth tube gradually separates from the gynoeceum tissue. Into this protuberance the bundles of the re-formed vascular ring fan out right and left (Fig. 93). At the same time the protuberance becomes twofold owing to a median indentation, half the bundles being distributed to each developing ovary (Fig. 94). As might be expected the amount of discarded vascular tissue is here almost nil. In the now separate ovaries (Figs. 95, 96) the line of cleavage develops between the paired bundles of each fertile carpel. At a higher level these latter bundles disappear, leaving only the dorsal cord and two wing bundles, as described for the normal flower. In a mature fruit from which the outer tissues have been removed so as to expose the endocarp, the limits of the two carpels can often be clearly seen; the fertile carpel cord encased in sclerenchyma forms a distinct keel along the one edge, separated by a furrow on either side from the folded valve carpel forming the rest of the nut, the midrib (dorsal cord) and secondary veins of which show at the base of the fruit as so many round scars or holes (Fig. 97).

Prunus Armeniaca, L., Apricot. The general course of events in this species is the same as in the Almond, and further detailed description is unnecessary.

Prunus pseudocerasus, Lindl. (Figs. 98-100). This species is distinguished by the fact that the wing bundles, forming the only secondary veins of the sterile carpel, are situated a considerable distance apart, so that they and the midrib (dorsal cord) mark off the vascular ring into three nearly equal arcs. This position and the further fact that a considerable stretch of vascular tissue occurs between them are irreconcilable with the old view that these wing bundles represent the marginal veins of a single fertile valve carpel. An unusually large quantity of vascular tissue enters the pith involving the whole, or almost the whole, of the arc between each wing cord and the dorsal cord, and all of that lying between the two wing cords themselves, except the portion destined for the fertile carpel cord.

Prunus Padus, L., Bird Cherry. Here also the vascular system is much reduced. So much of the xylem is utilized to form the perianth cords that after they have left the central ring all but some six of the residual bundle masses appear to consist wholly of phloem. Before any indication of the boundaries of the ovary is apparent, these six xylem-containing masses have already become distinguishable as the dorsal (sterile) midrib, a small secondary vein in either side, the two large wing bundles,

72. The flower stalk. 73. The flower base; the perianth cords have emerged, and the vascular ring has re-formed. 74-8. Successive stages in the formation of the terminal functional ovary. In 78 the inner face of the flower tube shows to right and left a region with a ragged contour indicating the place of attachment of the two supernumerary non-functional ovaries. 79. The base of one of these non-functional ovaries and the whole length of the other are seen in longitudinal section. 80. The style, A of the functional, B and C of the supernumerary ovaries.



FIGS. 81-100. [All from sections taken at successively higher levels except 97.] 81-97. *Prunus Amygdalus*, Stokes. 81. The flower stalk. 82. The flower base; the perianth cords have just left the vascular ring. 83. One side of the ovary is now free from the flower tube; the dorsal cord can be identified in the re-formed vascular ring. 84-6, 89-91. Later stages in the development

and between them the fertile cord. As the ovary becomes free from the perianth tube, the pith area is entirely occupied by discarded phloem. The fertile cord divides in two unusually early, the resultant halves, owing possibly to limitation of space, becoming confluent with the neighbouring strong wing bundles of the sterile carpel.

Prunus avium, L., Gean, Wild Cherry (Figs. 101-13 and 119-28). This species offers particularly favourable material for the study of the origin of the vascular system of the two carpels, for, owing to the considerable amount of pith present, the bundles of the re-formed vascular ring are distributed in a fairly large circle, and are therefore more easily identified. Furthermore, it is one of the species in the genus having a double-flowered form, and these double-flowered varieties provide the strongest confirmation of the bicarpellary constitution of the ovary. In a section of the normal single flower, taken just above the level of emergence of the perianth cords, ten distinct bundle masses are seen to be left behind, disposed on the alternate radii. Each of these masses shows a large phloem component, obviously double, and two small xylem strands (Figs. 101, 102). At a higher level some of these xylem strands come to an end. Of those that persist, one pair, with the addition sometimes of a strand from an adjacent pair, becomes consolidated into the dorsal cord; two opposite pairs, each again sometimes with the addition of a strand from an adjacent pair, become the well-developed wing bundles; while a pair lying between these two wing cords, and somewhat sunk in the pith, becomes the fertile cord (Fig. 103). Those remnants of the remaining pairs which do not wander into the pith—now replaced almost entirely by discarded vascular tissue—furnish one or two secondary veins (Fig. 104), and further development then follows the usual course.

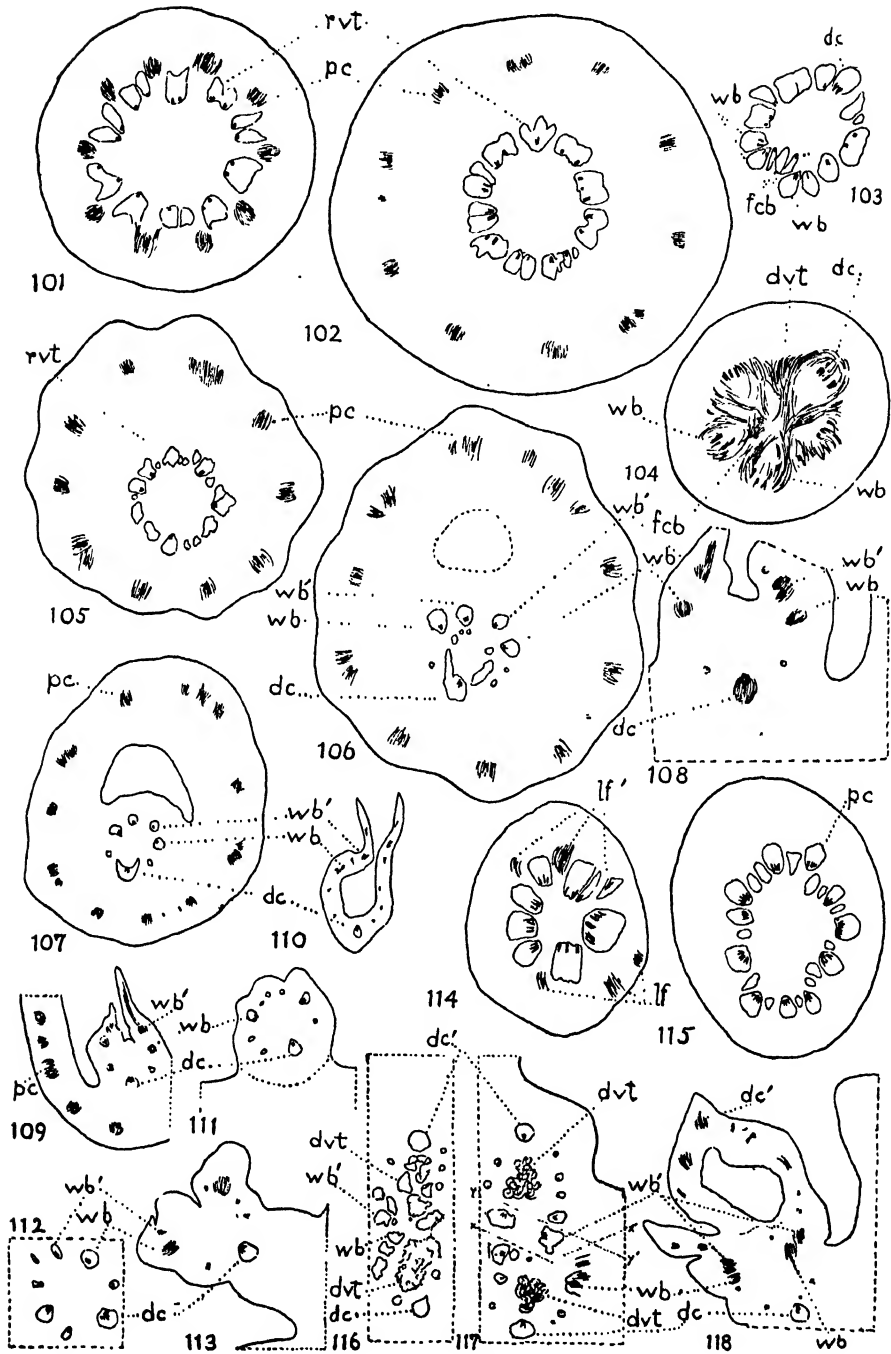
In the double variety the flower is often described as showing a folded green leaf in the position normally occupied by the ovary,¹ a fact which has been looked upon as tantamount to proof that the ovary, which it replaces, is composed of a single carpel. But examination of a considerable number of specimens shows that *two* such foliage-like leaves are quite often present, as has indeed been previously noted by Masters² and Worsdell,³ the outer enfolding the inner, and both being prolonged upwards into a style with terminal stigma (Fig. 127). These reversionary leaf-structures, it may be

of the ovary. 87, 88. Two stages in the development of the placental bundles, from another flower. 92-6. From a flower with two equivalent ovaries. 92. The flower stalk. 93-6. Stages in ovary development comparable with those shown for the single ovary in 82-6. 97. The ripe fruit after removal of epicarp and mesocarp seen obliquely from below. The fertile carpel forms a well-marked keel; the ring of dots indicates the broken ends of the veins of the sterile carpel. 98-100. *P. pseudocerasus*, Lindl. 98. Early stage in the differentiation of the vascular system of the sterile carpel; the wing bundles are placed far apart, the intervening space being occupied by vascular tissue, part of which will be utilized to form the fertile carpel. 99, 100. Later stages in the development of the ovary.

¹ See e. g. Asa Gray, Structural Botany, p. 172.

² Vegetable Teratology, p. 256.

³ Principles of Teratology, Pl. XXXVIII, Fig. 1, and vol. ii, pp. 93 and 194.



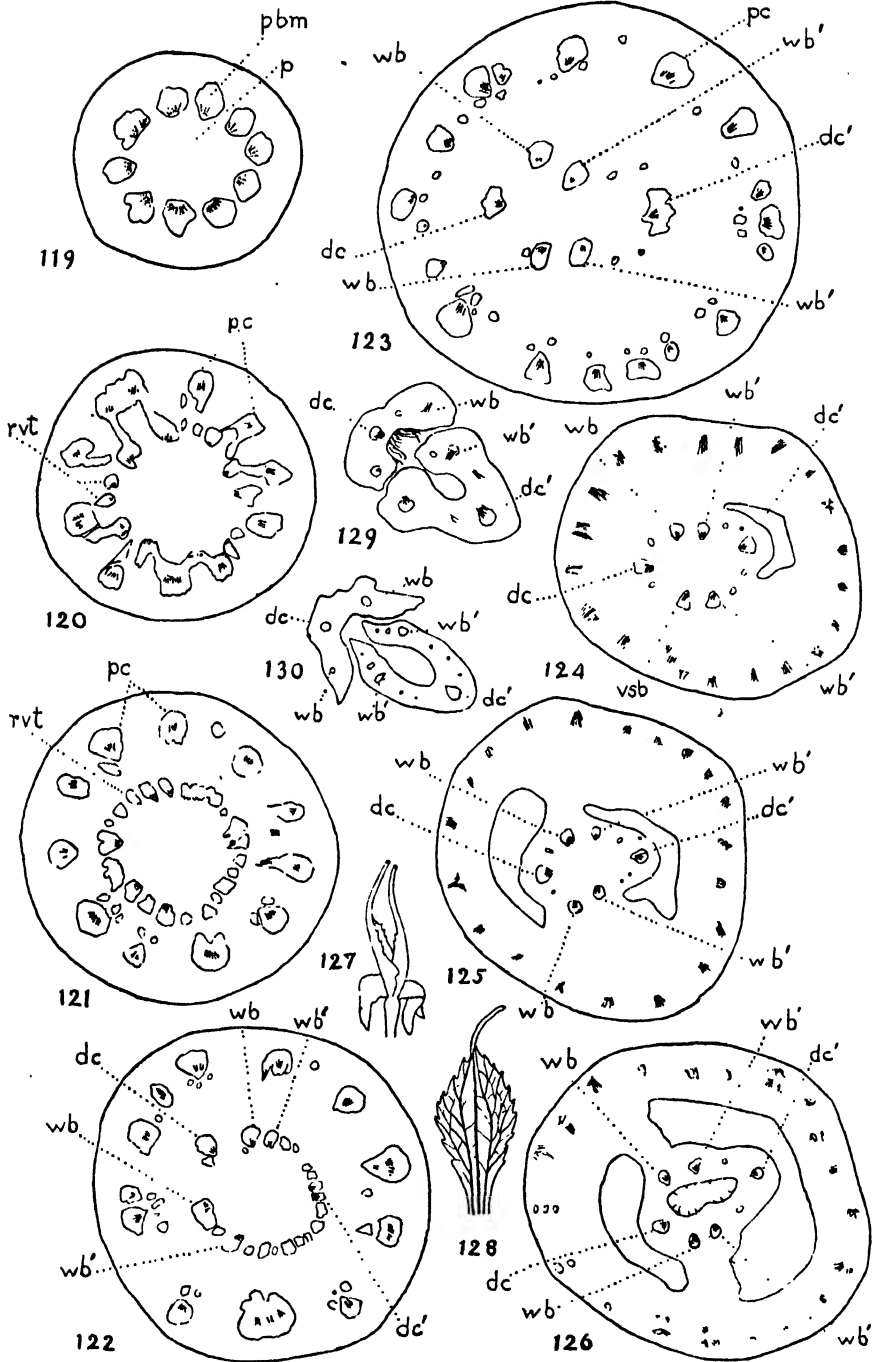
FIGS. 101-18. [All from transverse sections taken at successively higher levels.] 101-4. *Prunus avium*, L. (single). 101. The flower base; the perianth cords are emerging from the ring. 102. The residual vascular tissue has been re-formed into a ring. 103. The re-formed ring is becom-

observed, do not exhibit a strong marginal vein such as might have been looked for were they in truth the sterile equivalents of whole ovaries composed of a single valve carpel (Fig. 128). Instead, they show three strongly developed veins in the central region which, although the lamina here is sessile, no doubt correspond with the three bundles which leave the axial ring separately in the case of the normal foliage leaf (Fig. 114), but which in the latter case fuse shortly to form the single cord of the petiole and the midrib of the blade.

Sections taken through the base of the double flower show the usual mode of origin of the vascular cords for the perianth (Figs. 119, 120). These cords having passed out from the vascular ring, further development direct from the axis depends on the number and size of the xylem strands left behind and available for further construction. This is shown in Figs. 105, 106, and 121-3, and is illustrated further by diagrams (p. 597) representing the various arrangements of this residual vascular tissue found in the specimens examined.

The arrangement shown in A indicates the presence of two more or less similar leaves, one enfolding the other and each furnished with a prominent midrib and wing bundles (cf. Figs. 124-7, 129, 130). Scheme B shows the appearance seen when only the first leaf attains full development, the second, supplied by three small bundles (Fig. 111), being often membranous and nearly colourless, and rounded at the apex, which shows no hint of a style. In the third case, C, only one leaf is formed; the bundles corresponding to the second leaf are too feeble to allow of full development, and the convex outline over the centre bundle (midrib) is all that we find to represent the second member, the two flanking bundles entering the first leaf, where they run peripherally to its own proper wing bundles (Figs. 112, 113). In the last example, D, there is again only a single leaf. The median bundle of the second leaf is here wanting altogether; the strong wing bundles are deflected, as in the previous case, into the first leaf, which thus shows a double set (Figs. 105-10). It is clear that the first leaf is the counterpart of the sterile valve carpel of the 2-carpelled ovary. The second leaf member, corresponding to the fertile solid carpel, may be partially or completely suppressed as in

ing differentiated into the dorsal, wing, and fertile bundles. 104. A large quantity of phloem is discarded. 105-13. *P. avium* (double). 105-10 from flower A. 105. Stage corresponding to that shown for the single flower in 102. 106-10. The re-formed vascular ring becomes differentiated into dorsal and wing bundles on the one side, and into wing bundles without a dorsal cord on the other, with the result that the ovary is replaced by a single leaf. 111-13 from another flower B. Both the dorsal and wing cords of a second leaf are present, but they are so feebly developed that the leaf which they should supply is not formed, and as in flower A the ovary is replaced by a single leaf. 114-18. *P. Cerasus*, L. 114. Apex of young vegetative shoot showing the mode of origin from the axial vascular ring of the midrib and the two main secondary veins of two foliage leaves. 115-18. *P. Cerasus* (double). 115. The flower base at the level at which the perianth cords begin to emerge from the vascular cylinder. 116, 117. The re-formed vascular cylinder of the same flower undergoing bifurcation (xx'), the line symmetrically dividing the bundles of the two leaves; yy' , the actual line of separation). 118. One of the two leaves replacing an ovary is becoming disjoined from the other.



FIGS. 119-30. *Prunus avium* (double) (continued). [All from transverse sections taken at successively higher levels except 127 and 128.] 119. The flower stalk. 120. The flower base at the

schemes C and D, or it may show a more or less complete return to a vegetative character as in the cases illustrated in A and B. Development is directly in accord with the amount of residual vascular tissue present, all of which is utilized.

Similar appearances to those described are to be seen in the double varieties of *P. japonica*, Thunb., and *P. Cerasus*, L., but in the latter species the doubling is often of an extremely complex nature, several supernumerary pairs of style-bearing green leaves being present in some flowers in addition to the central pair (Fig. 131). These extra pairs are found to arise in secondary florets (Fig. 132), formed from buds developed among the numerous

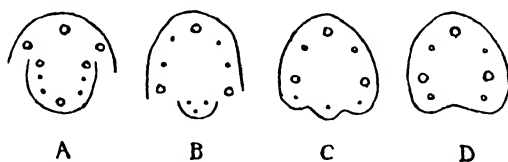
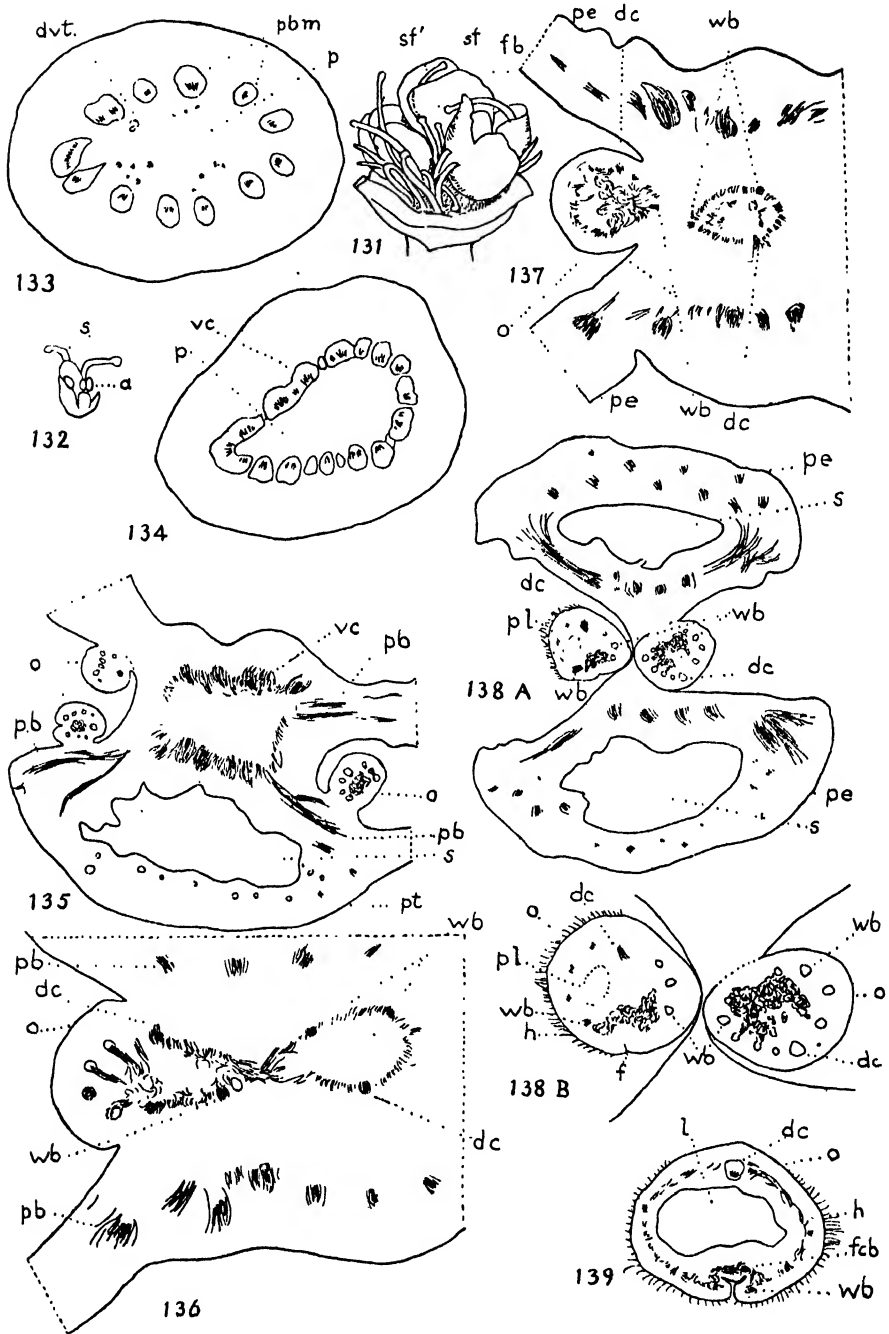


FIG. 130 A. For description see text (p. 595).

petals. The manner of origin of the vascular system of the central pair is traced in Figs. 115–18. Fig. 115 shows the appearance seen at the level at which the perianth cords begin to pass out from the central cylinder. At successively higher levels (Figs. 116, 117) two oppositely placed dorsal cords and two pairs of strongly developed wing bundles gradually become differentiated, following upon a flattening out of the vascular ring, preparatory to division across the narrow diameter into two roughly equal groups of bundles. In both groups a certain amount of vascular tissue enters the central parenchyma and there comes to an end. This discarded tissue is comparable in origin and position with that from which the fertile cord (carpel) of the ovary of the single flower is derived. There evidently occurs here a ‘twinning’ of the vascular cylinder, such as has already been described for ‘otherwise normal flowers in the Victoria Plum and Almond (see pp. 585 and 591 and Figs. 70, 71, 94, 95). But in place of the two bicarpellary ovaries composed each of a sterile and a fertile carpel present in this type of single flower we have in the double *P. Cerasus* only the two leaves corresponding to the two sterile carpels. The residual vascular elements in each half of the axis, from which the second (fertile) carpel is derived in the case of the single flower, are here discarded in their entirety. Hence notwithstanding the bifurcation of the axis cylinder only two leaves are developed. They represent the normally sterile member in each of two ovaries, whereas in *P. avium*, as described above, the two leaves correspond to the two carpels

level of origin of the perianth cords. 121. The vascular ring has been re-formed. 122–6. The re-formed vascular ring becomes differentiated into the well-developed dorsal and wing cords of two oppositely placed leaves. 127. The flower after part of the calyx has been cut away and the petals removed; in the centre two folded leaves with styles which replace the ovary. 128. One of these two leaves in surface view (more highly magnified). 129, 130. The two leaves now free from the flower tube and from each other, showing dorsal and wing cords; the edges of the one leaf enfold those of the other.



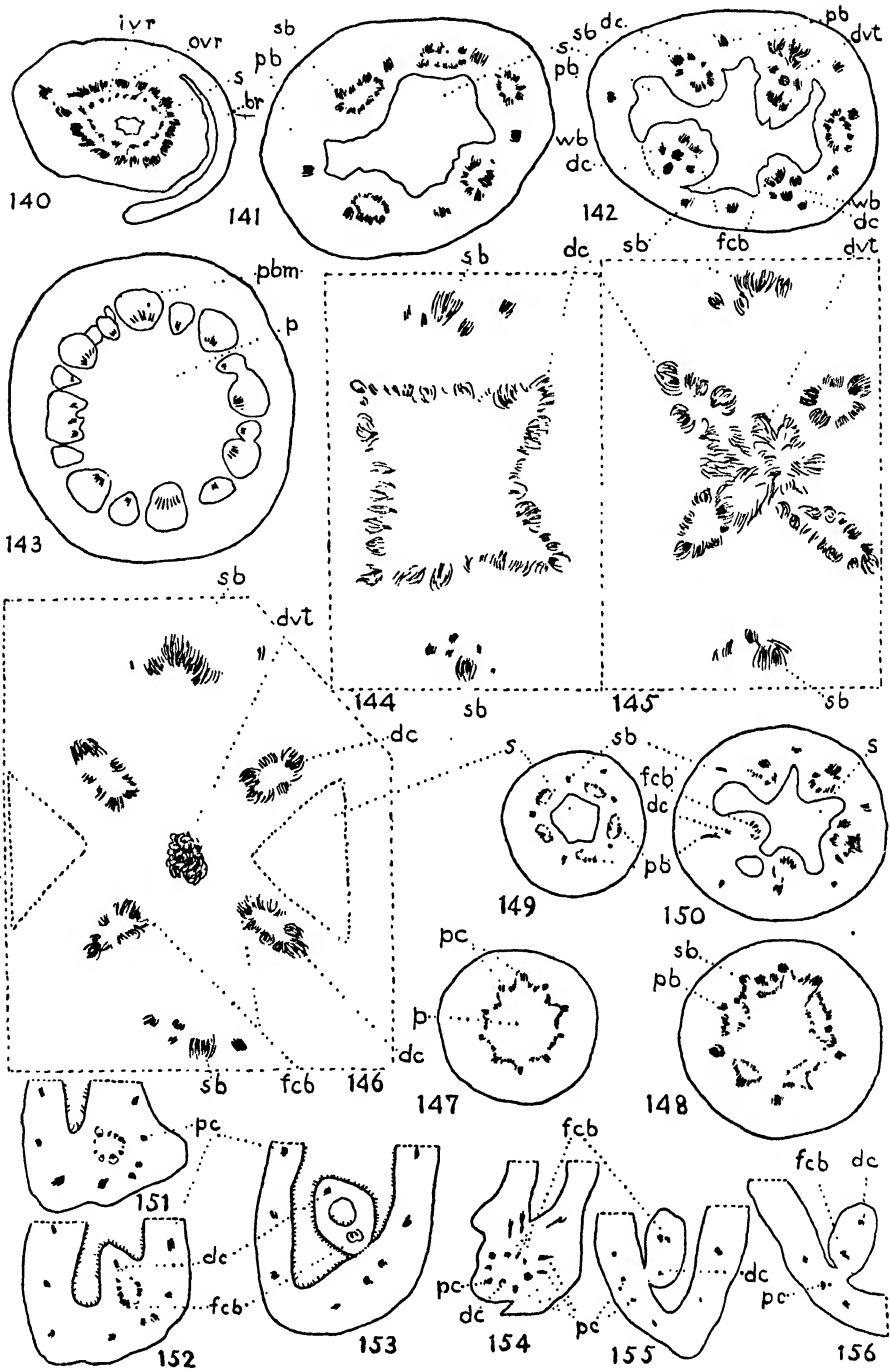
FIGS. 131-9. 131, 132. *Prunus Cerasus* (double)(continued).¹ 131. A flower with the perianth cut away to expose the secondary florets, some of which show two naked styled leaves, others

¹ See Figs. 115-18.

of an individual ovary. It is a somewhat curious fact that although the vascular tissue in the bifurcate axis is divided into two equal halves, and although the two pairs of wing bundles are symmetrically distributed on either side of the dividing line, it sometimes happens that the line of separation of the two leaves does not coincide with the line passing between the two pairs of wing bundles, but cuts across it in such a way as to leave all four bundles on the one side. It results that in the one leaf there are two of these cords in each half of the lamina, while in the other there are none. The process of reversion, though complete up to a point, is not perfectly adjusted to the normal mode of development. In the process of separation the midrib of the first leaf to become free bends outwards so that the two halves of the lamina are drawn farther and farther from the axis until severance of the margins by narrowing of the connecting tissue is finally effected. It would seem as though the position of the line of disjunction were to some extent dependent on mechanical strain the incidence of which did not necessarily bear a strict relation to the anatomical line of division.

Prunus triloba, Lindl. (Figs. 133-9). This is another species with a double-flowered variety, but here, notwithstanding the large number of tightly packed petals, normal ovaries are formed sometimes to the number of seven or eight in one flower, one or occasionally two arising from the stem apex, the others being borne in a ring on the sides of the flower. A cross-section of the flower stalk taken from a specimen with two such centrally placed ovaries shows some ten or more primary bundle masses (Fig. 133) which become condensed into a continuous ring (Fig. 134). After the emergence of the perianth cords the ring becomes constricted and finally halved (Figs. 135-7). The two secondary rings formed by this process of 'twinning' supply the two central ovaries. In each of these rings one can already identify, at points almost equidistant from one another, the three xylem-containing bundles which will furnish the midrib and the two strong wing bundles of the sterile carpel (Figs. 136, 137), while within the ring is to be seen a considerable amount of residual vascular tissue (Fig. 137), some of which is utilized in the formation of the cord of the fertile carpel (Figs. 138 A, B). When the vascular cylinder of the axis remains undivided only a single central ovary is formed, the mode of development and plan of construction being the same as in the case of twin ovaries. Those borne on

a similar pair of leaves enclosed in their own perianth (*sf'*); in the centre the pair of styled leaves proper to the main flower (*sf*). 132. A secondary floret. 133-9. *P. triloba* (double). 133. The flower stalk. 134. The same; the primary bundle masses have broken up into smaller bundles preparatory to the differentiation of the perianth cords. 135-8. Stages in the bifurcation of the axial vascular cylinder previous to the formation of two central ovaries. 135. Three accessory ovaries have been formed from buds on the wall of the flower. 136, 137. The two wing bundles of the sterile carpel are widely separated, the intervening space being occupied by an arc of vascular tissue. 138 A. The sterile carpel of the central ovaries is now fully differentiated, and the residual vascular tissue is becoming concentrated to form the cord of the fertile carpel. 138 B. The two central ovaries more highly magnified. 139. An ovary showing exceptional development of the fertile carpel, which appears as a bulge beneath the contiguous edges of the folded sterile carpel.



FIGS. 140-56. [All from transverse sections taken at successively higher levels.] 140-2. *Nuttallia cerasiformis*, Torr. and Gr. 140. The flower base with bract becoming detached. The

the walls of the flower also arise in the same fashion. Yet one more piece of evidence supporting the 2-carpel interpretation was afforded by an individual ovary in which the edges of the folded lamina of the sterile carpel scarcely met, so that a definite passage rather than a mere line of cleavage appeared between them (Fig. 139). Blocking the inner end of this passage was an undivided median hump of tissue surrounding the fertile cord of the other carpel. The interest of this case lies in the fact that it is rare to find any trace of the carpel boundaries or any considerable extent of tissue over and above the fertile cord belonging to the second carpel. As a rule the cleavage line separating the margins of the sterile carpel extends uninterruptedly between the paired bundles of the fertile carpel right up to the loculus, splitting the inner member in half at quite an early stage.

Sufficient evidence has been brought forward in the above account of a number of species of *Prunus* to justify the following statement of the position. On the supposition that the normal ovary is composed of *two* carpels, the whole of the facts adduced fit together and form an intelligible whole. On the orthodox view that $G = 1$ they can neither be reconciled nor explained, they remain as so many anomalies.

Nuttallia cerasiformis, Torr. and Gr. (Figs. 140-2). In this monotypic genus we meet with a new condition which has a considerable effect on the mode of development of the gynoecium. The stem apex is markedly concave and begins to become so at a level below that at which the vascular cords for the perianth are differentiated from the rest of the central vascular ring (Fig. 140). From this level upwards there is therefore a free surface on the inside as well as on the outside of the wall of tissue, from which the perianth whorls are presently exerted, and from which the ovaries arise as protuberances with the ventral surface exposed and projecting into the concavity. Before there is yet any sign of this central space a second ring of vascular elements makes its appearance within the original one. This inner ring is derived from the outer through the giving off centrewards of elements of both xylem and phloem (Fig. 140) which show a reversed orientation, the xylem being towards the outside, the phloem nearer to the

vascular tissue forms an outer and an inner ring, the latter surrounding a space, the axis having already become concave. 141. The five simple cords of the petals are now differentiated, the remainder of the vascular tissue forming five alternating secondary rings which will furnish the sepal cords and the sterile and fertile carpel bundles. 142. The five developing ovaries form five protuberances on the inner wall of the flower. 143-6. *Rhodotypos kerrioides*, Sieb. and Zucc. 143. The flower stalk. 144. The vascular ring has re-formed after the emergence of the perianth cords. 145, 146. Stages in the rearrangement of the vascular tissue to form the cords of the sterile and fertile carpels. 147, 148. *Spiraea ulmifolia*, Scop. 147. Earliest stage in the differentiation of the perianth cords. 148. Formation of the sepal cords and of the alternating secondary rings which furnish the petal cords and the sterile and fertile carpel cords. 149, 150. *Spiraea chamaedrifolia*, var. *subracemosa*. 149. The same stage as shown in 148, but in this species the axis has already become concave. 150. The five ovaries appear as protuberances on the inner face of the flower wall; two have developed in such close proximity as to have become joined laterally. 151-3. *Stephanandra Tanakae*, Franch. and Sav. 154-6. *S. flexuosa*, Sieb. and Zucc. Stages in the development of the solitary ovary which arises as a protuberance on one side of the flower tube.

centre. At a slightly higher level the concentric ring arrangement disappears, the bulk of the vascular elements are concentrated into five smaller secondary rings on the five radii of the sepals, while the remainder form a simple cord on each of the alternate radii (Fig. 141). These latter cords become the midribs of the petals. Of the bundles forming each of the small rings, one, situated on the outer curve, sometimes near the mid-point, sometimes farther to the right or left, passes outwards to become a sepal midrib. A few elements, mostly phloem, wander into the enclosed area of parenchyma and are discarded. The remainder of those situated on the outer curve give rise to the midrib and strong wing bundles of a sterile carpel, while those on the inner half of the ring form twin fertile bundles (Fig 142). In consequence of the varying and often asymmetric position in the outer arc of the sepal cord, and hence of the sterile carpel (= dorsal cord), it comes about that the ovaries are frequently not centred strictly in line with the sepals, though standing more or less in front of them. This variability probably accounts for the conflicting statements of different writers, Eichler and Baillon describing the ovaries as opposite the petals, while Juel¹ places them more correctly opposite the sepals.

We find very much the same arrangement of the vascular tissue as that described above in species of *Spiraea* (see later, p. 605), and the underlying cause is probably the same in both cases. As the result, apparently, of the configuration of the stem apex, which presents a free surface on both sides of the wall of tissue from which the floral whorls arise, the reconstruction preparatory to carpel formation proceeds centripetally. But there is here no central area of parenchyma in which the necessary reconstitution of the residual vascular tissue can take place, and a rearrangement at a lower level where there is still complete tissue continuity is necessitated, resulting in the formation of the small secondary vascular rings, each of which consists of the elements to be used in forming a sepal midrib and the sterile and fertile cords of an ovary. Had we no other example besides *Nuttallia* of this kind of construction, it would have been difficult to arrive at an understanding of these relations, but the knowledge gained from a study of those species of *Spiraea* in which the axis is similarly concave leads to the conclusion that the vascular elements which, in a type with a distinctly convex stem apex, would re-form on the radius, and to the inside of a sepal, in *Nuttallia*, become differentiated as the dorsal cord and wing bundles of a sterile carpel *before the true radial position has been assumed*, while the elements which should re-form on the radius of the petal never concentrate as a cord in this position at all. Instead they diverge prematurely to form the inner arc of the secondary rings, and eventually become the fertile bundles. It would seem that the considerable hollowing out of the axis has led to a shortening of the process of ovary formation by the elimination

¹ Loc. cit., p. 10.

of this preliminary step in the formation of the inner carpel whorl. If the comparison with *Spiraea*, in which this rearrangement is more easily followed, is well founded, the two fertile bundles are to be regarded as homologous with those of two half carpels, which, in a corresponding type with a convex stem apex, would constitute, respectively, half of the carpel in line with the petal to right and to left. This construction distinguishes *Nuttallia* from *Prunus* and brings it into line with most other Rosaceae in which the ovaries are isomerous with the perianth whorls, $\frac{1}{2} \text{ } 1 \frac{1}{2}$ carpels being the usual plan of construction of each ovary in these cases.

Kerrieae (Figs. 143-6, 166-8, 197-9).

Rhodotypus kerrioides, Sieb. and Zucc. (Figs. 143-6). The flower, usually tetramerous, though occasionally pentamerous, shows a stem apex broad and nearly flat, the ovaries becoming free, in consequence, almost simultaneously from perianth wall and central pith. In transverse section the top of the flower stalk shows an abundant pith and a roughly 4-sided vascular ring having a well-marked bundle in the middle of each side (orthogonal) and one at each angle (diagonal) (Fig. 143). The intervals between these eight bundles are occupied by more irregular masses completing the ring. The orthogonal and diagonal bundles pass outwards as the perianth cords. The residual portions then close up so that a continuous ring is again made (Fig. 144). A new bundle is reconstituted on each of the diagonal radii (= those of the petals), and these four new bundles furnish the dorsal cord of each of the four ovaries. No reconcentration of xylem tissue takes place on the orthogonal (sepal) radii after the emergence of the sepal cords. Some of the residual elements, chiefly phloem, wander into the pith (Fig. 145). The remaining groups lying on the radius between sepal and petal move inwards, converging in pairs, the two groups on each side of a petal thus coming together to the inside of and in line with the petal and dorsal cords, and forming the twin fertile bundles of the ovary (Fig. 146). These appearances suggest that here again the ovaries are constructed upon a $\frac{1}{2} \text{ } 1 \frac{1}{2}$ carpel scheme, but that a modification of the usual ground-plan is necessitated by the flattened form of the stem apex, which does not afford sufficient vertical height for the successive reconstitution of two whorls of carpel cords in the ordinary way. Hence, although the amount of vascular tissue utilized may be regarded as equivalent to one whole cord and two half cords (carpels), the elements of the half cords, being the portions of the ring left between the radius of sepal and petal, are not actually re-formed into a single whole cord, but diverge prematurely, swinging round to take up their position to the inside of, and facing the dorsal cord. As in *Nuttallia* the process of ovary formation is hurried up by the elimination of this intermediate step. The reason for the unusual position of the ovaries opposite the petals is not self-evident, unless it be that their dis-

position as well as their construction is governed by the time factor. The outgoing sepal cords have carried with them all the xylem elements on these radii. In these circumstances reconcentration on the line of the sepals would involve the coming together of elements which are a considerable distance apart, whereas the formation of a dorsal cord in line with the petals can be immediately effected, since the necessary elements are close at hand. This procedure would be quite in keeping with the other time-saving modification mentioned above, and there seems no other obvious reason for this departure from the rule of alternating whorls.

Kerria japonica, DC. (Figs. 197-9). In the normal flower there are five rounded egg-shaped ovaries, each bearing a style a little below the apex on the ventral side (Fig. 197). As described by Eichler¹ and Baillon² and contrary to Juel,³ who follows Maximovicz, the ovaries stand, as in most Rosaceae, in line with the sepals. They arise as protuberances around the very slightly convex stem apex, the dorsal side becoming free from the flower wall, while the ventral side is still undefined and continuous with the last remnant of pith. A strand from the central vascular cylinder passes out into each protuberance, running first horizontally and then upwards to form the dorsal cord (sterile carpel). As in *Rhodotypus* and in *Spiraea* (described below) the process of ovary formation is shortened by the omission of the stage of reconstitution into whole cords of the residual vascular tissue, destined for the inner carpel whorl. The residual tissue lying between two adjacent sepal radii is condensed prematurely into two portions which diverge and swing round to right and left. It thus comes about that a half portion (= half carpel) on each side of a sepal cord, converges to the inside of the dorsal cord, the two forming the two ventral bundles in each ovary. During this rearrangement a branch from the dorsal cord connects with each of the two fertile bundles, a feature not observed in any of the genera previously treated. These branches would appear to represent the sterile carpel wing bundles of other genera. Only a single ovule is formed, which is supplied by a strand from one of the fertile bundles, both strand and ovule taking up a median position. At the top the dorsal cord sweeps inwards over the shoulder of the ovary and enters the laterally placed style together with the two ventral bundles, all three persisting till the stigma region is reached.⁴ The condensation of the wing and fertile bundles into single strands makes it difficult to determine whether both are prolonged upwards in these strands entering the style, but from the fact that the style forms a direct continuation of these strands, and from the appearances seen in a double flower, this would seem probable.

¹ Blüthendiagramme, p. 509.

² Nat. Hist., vol. i, p. 380.

³ Loc. cit., p. 28.

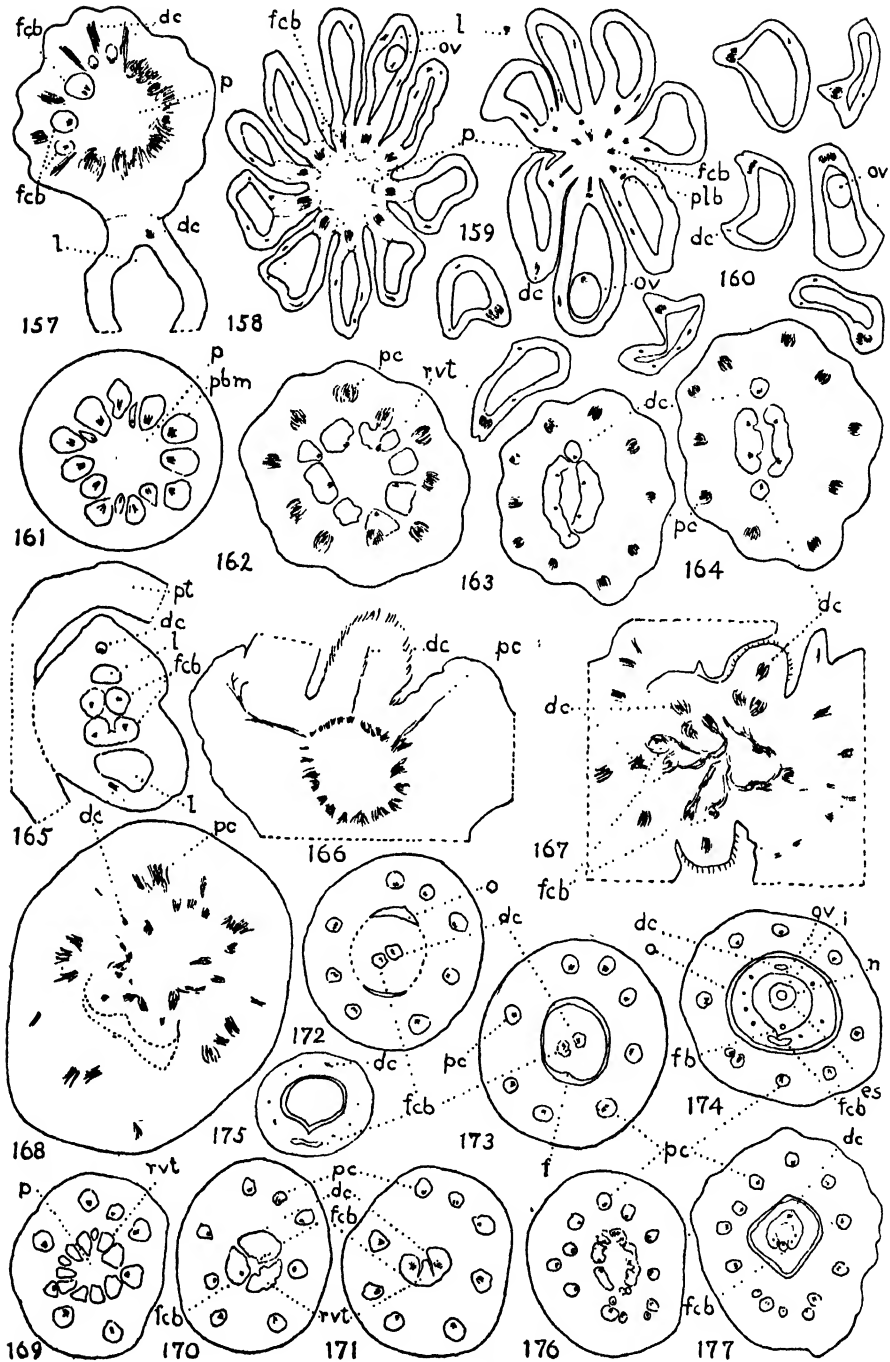
⁴ Juel states (loc. cit., p. 34) that midway up the style the dorsal cord is either lacking or pushed to one side; this was not the case in the specimens which I examined.

From the present point of view the double flower is of great interest, for it also, despite the doubling (due to petalody of the stamens), possesses five ovaries, but they are entirely different in shape from those of the single flower and have a *terminal* style and *numerous* ovules (Figs. 198, 199). The vascular supply of the follicle-like ovaries is derived in the same manner as in those of the single flower, but in the double the ventral fertile bundles soon come to an end. Above this point the normally sterile outer carpel becomes fertile, producing several true marginal ovules which are supplied by the wing bundles. At a higher level the wing bundles in their turn cease, and from here upwards the ovary remains open in the ventral line. The midrib, which now alone persists, is prolonged upwards into the style, which here continues the lines of the dorsal cord, whereas in the single flower it springs from the ventral side. These differences between the gynoeceium of the single and the double are wholly inexplicable on the current view that the normal ovary is monocarpellary.

Neviusia alabamensis, A. Gray (Figs. 166–8). The only feature in *Neviusia* which calls for remark is the absence of a corolla; in consequence the ovaries alternate with the sepals. Otherwise in mode of origin and structure they resemble those of *Kerria*.

Spiraeaceae (Figs. 147–65, 180–2).

Spiraea spp. (*S. ulmifolia*, Scop., *S. chamaedrifolia* var. *subracemosa*, *S. Henryi*, Hemsl., *S. Sargentiana*, Rehd., *S. Thunbergii*, Sieb., *S. japonica*, L., *Aruncus silvester*, Kostel (Figs. 147–50): As already stated (p. 602) the stem apex in many species of *Spiraea* (used in the narrow sense) is distinctly concave, the ovaries being then seated on the flower wall as in *Nuttallia*, projecting therefrom into the central concavity as they develop. In these forms the ventral surface of the ovary becomes free first, the dorsal last. Other species with a more shallow concavity show a low mound of tissue in the centre. In these types the ovaries become exposed first along the two sides, then almost simultaneously, as the pith disappears, on the dorsal and ventral faces, as in *Rhodotypus*. That is to say, there occurs in this genus, in greater or lesser degree, that shortening of the axis which, as we have seen in *Nuttallia*, *Rhodotypus*, *Kerria*, and *Neviusia*, appears to have a far-reaching effect on the mode of origin of the carpels. The ovaries arise in line, or almost in line with the petals. Why the law of alternation is not followed here is not clear. The bundles which will emerge to form the perianth midribs can usually be identified while the axial vascular ring is still unbroken and the central region still occupied by pith (Fig. 147), but those which are destined for the sepals are not so large as to suggest that the size of the resulting gap is the reason why no inner cord is re-formed on this radius, and the cause of this anomaly is still to seek. In the forms with a markedly concave axis the arrangement of the vascular tissue follows

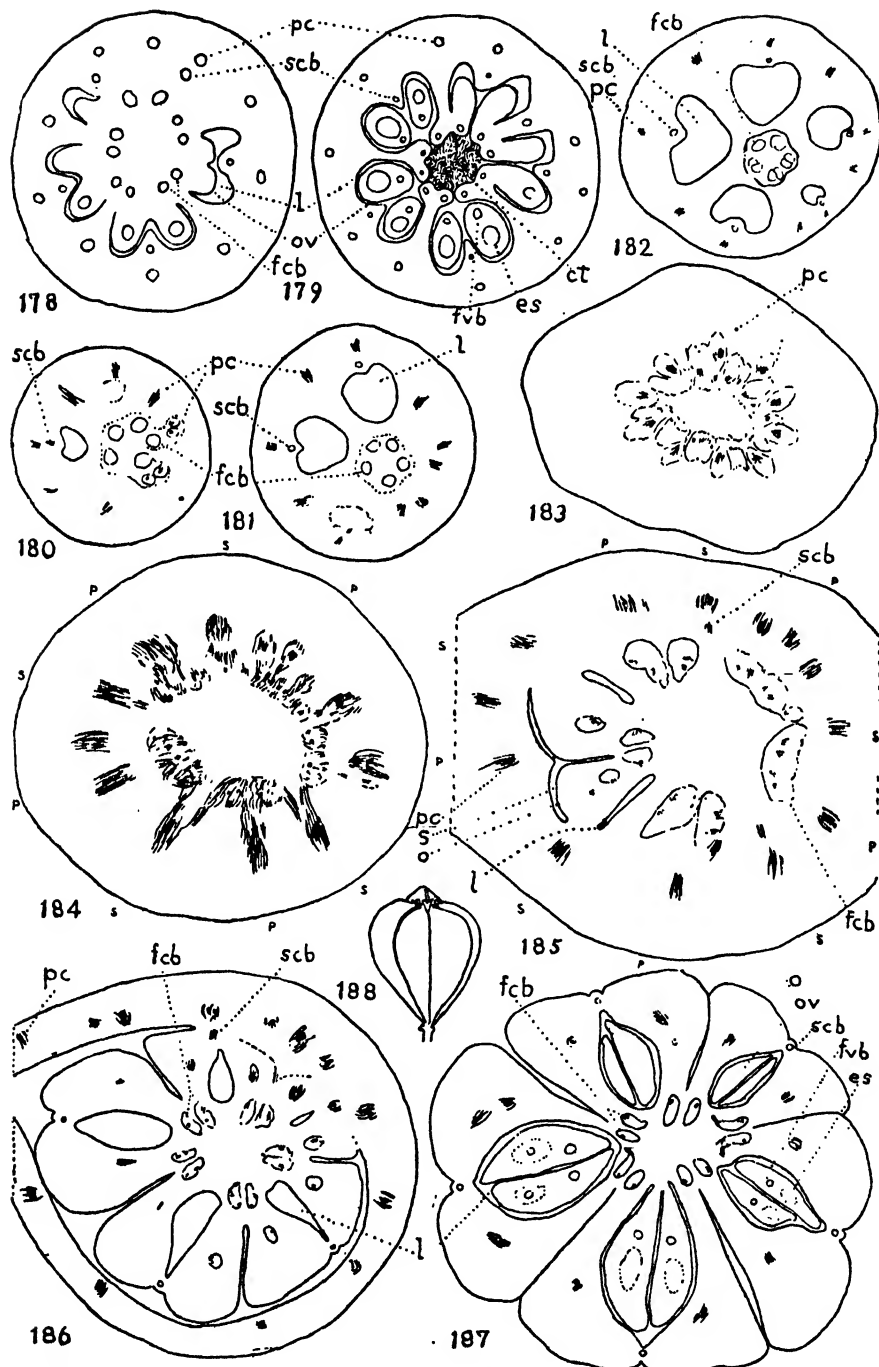


FIGS. 157-77. [All from transverse sections taken at successively higher levels.] 157, 158. *Filipendula Filipendula*, Voss. (*Spiraea Filipendula*). 157. The base of the gynoecium. On the left the dorsal cords have just left the central ring; elsewhere they have already passed out along the

the same course as in *Nuttallia*, except for the change of radius, the secondary rings here being opposite the petals (Figs. 148-50). In some species the petal cord and the dorsal cord of the ovary emerge separately from these secondary rings, in others the two pass out as one strand and separate later. The twin fertile bundles of each ovary are formed from the inner arc of a secondary ring, which in turn is derived from the residual portions of the original ring lying on the intermediate radii between any petal and the sepal on either side. We may conclude that here, as in the other genera with a flat or concave axis already described, the material out of which each ovary is constructed is equivalent to $\frac{1}{2} \times 1\frac{1}{2}$ carpels, but that the stage of reconstituting the vascular tissue of the inner carpel whorl into whole cords is eliminated, thereby enabling development to proceed more rapidly.

Stephanandra (Figs. 151-6). In this genus, which is grouped with the true *Spiraeas*, there is usually but a single ovary, which arises from one side of the flower tube. Transverse sections show the same mode of construction as has been described for those members of the *Sanguisorbeae* which have a solitary pistil. After the emergence of the perianth cords, the residual vascular tissue forms a more or less complete ring, consisting mainly of phloem, the xylem elements being concentrated at two opposite points on the circle. These become respectively the dorsal and fertile cords, representing two whole carpels. Here, as in *Prunus* and many of the *Sanguisorbeae*, the position of the ovary is readily explained on the present interpretation, whereas on the orthodox view (that $G = 1$) we meet with the old difficulty of a terminal leaf-structure. In the specimen of *S. Tanakae*, Franch. and Sav., here represented (Figs. 151-3) the dorsal surface of the ovary first projects freely into the flower tube, while the tissue of the ventral side is still continuous with it and undelimited (Fig. 152). In *S. flexuosa*, Sieb. and Zucc., this was the case with one flower (Fig. 156); in another the

lateral walls of the ovaries, all of which, except one (partly shown), have become detached. 158. The gynoecium at the level of attachment of the ovaries, some of which show twisting. The dorsal cord is seen in all stages of its passage from the central vascular ring to the dorsal position. 159, 160. *Filipendula Ulmaria*, Maxim. (*Spiraea Ulmaria*). 159. A gynoecium of seven ovaries, some of which are twisted. 160. Individual carpels showing various degrees of contortion. 161-5. *Neillia opulifolia*, Benth. and Hook. 161. The flower stalk. 162. The perianth cords have just emerged, leaving a ring of residual bundles, of which only six appear to contain xylem. 163, 164. Stages in the differentiation of the two dorsal cords and in the halving of the two alternate fertile cords. 165. The gynoecium is becoming free from the perianth tube and is beginning to constrict to form two separate ovaries, in which the loculi have made their appearance. 166-8. *Nevisia alabamensis*, A. Gray. 166. A vascular bundle is leaving the central ring to become the dorsal cord of one of the five ovaries which appears as an oval protuberance. 167. Two ovaries have become free from the surrounding wall of the flower, part of which has been cut away in this and the preceding figure. A portion of the vascular tissue is becoming rearranged to form the carpel bundles, the rest being discarded. 168. A later stage from another flower. 169-75. *Cercocarpus betulaeifolius*, Nutt. 169. The flower base; the perianth cords have just left the central ring. 170. The residual vascular tissue consists of irregular masses, only two of which contain xylem. 171-3. Stages in the delimitation of the solitary ovary and in the formation of the vascular cords for the sterile and the fertile carpel. 174, 175. Further development and formation of the ovule ($n = nucellus$). 176, 177. *Purshia tridentata*, DC. 176. The flower base; the perianth cords have emerged, and the residual vascular tissue has re-formed into an irregular ring. 177. The residual vascular tissue has become concentrated into the dorsal and wing bundles of the sterile carpel and the cord of the fertile carpel.



FIGS. 178-88. [All from transverse sections taken at successively higher levels except 188.] 178, 179. *Amelanchier canadensis*, Torr. and Gray. 178. The flower base at the level of origin of the loculi, which are unequally developed from back to front. The five ovaries are more or less bi-

ventral side of the ovary faced the central axis and became exposed first, the dorsal still remaining for some time attached (Fig. 155). This variability in the way in which the ovary is orientated is another feature which is difficult to reconcile with a monocarpellary structure, since in one flower we must accept that the carpellary leaf turns its dorsal and in another its ventral side to the flower centre. But in an ovary composed of a surviving pair of carpels it may well be even chances whether the one or the other becomes detached first from the flower wall.

Filipendula (Figs. 157–60). The two species *F. Filipendula*, Voss. (*Spiraea Filipendula*, L., Dropwort), and *F. Ulmaria*, Maxim. (*Spiraea Ulmaria*, L., Meadow-sweet), formerly included under *Spiraea*, are distinguished from the true *Spiraeas* by having from 5 to 12 or 15 ovaries, each composed of two whole carpels. In *F. Filipendula* the ovaries (generally 10 or 12 in a single whorl), though not obviously twisted when the full number are present, frequently show some degree of distortion as development proceeds. In *F. Ulmaria*, where the ovaries are fewer (5–8), they undergo considerable twisting with consequent distortion of the loculus, often taking up a position more or less broadside on to the axis. This behaviour is the direct outcome of their mode of construction. After the emergence from the axial cylinder of the 10 or 12 cords destined for the perianth members, there pass out another whorl of vascular strands (10 or 12 in *F. Filipendula*, fewer in *F. Ulmaria*), one of which travels horizontally outwards in one side wall of each developing loculus (Fig. 157), until the periphery is reached, when it turns upwards, forming the dorsal cord (sterile carpel). The remainder of the vascular tissue becomes reconstituted as 10 or 12 or fewer bundle masses on the alternate radii; each bundle mass becomes differentiated into twin bundles, and each such pair of bundles forms the fertile cord of one ovary (Fig. 158). Thus every ovary is composed of an outer sterile carpel on one radius, and a fertile inner carpel standing on the adjacent radius. As the pairing of outer and inner carpel cords takes place in orderly fashion, all the way round the circle the ovaries are all turned slightly sideways in the same direction. In *F. Filipendula* the large number of ovaries prevents any considerable turning movement, but in *F. Ulmaria*, where the smaller number gives freer play, the twisting

locular owing to the incomplete withdrawal from the centre of the sterile carpels. 179. An older stage. 180–2. *Sorbaria sorbifolia*, A. Br. (*Spiraea Lindleyana*). Successive stages in the formation and emergence of the perianth cords, in the appearance of the sterile carpel bundles and loculi opposite the sepals, and in the halving of the fertile carpel bundles. Owing to the one-sided development of the flower, different stages are to be seen on the two sides of each figure. 183–8. *Exochorda Alberti*, Reg. 183. Earliest stage in the differentiation of the perianth cords. 184. Emergence of the perianth cords. (In this and the succeeding figure s = sepal radius, p = petal radius). 185. The dorsal cords which have been carried out conjoined with the sepal cords are becoming disjoined; the fertile carpel cords are becoming halved. 186. The gynoecium is becoming detached from the perianth; the sterile carpels are seen as small bulges in line with the loculi. 187. The gynoecium now free. A split in the mid-line of the fertile carpels leads to the formation of five wings. 188. The fruit.

is very marked (Figs. 159, 160). On the old view that the ovaries consist of a single carpel, this striking feature of the genus remained unexplained.

Neillia (Figs. 161–5). *Neillia opulifolia*, Benth. and Hook (formerly included in *Spiraea*), has two or in early flowers sometimes three ovaries, conjoined at the base, and showing with delightful clearness the $\frac{1}{2} \times \frac{1}{2}$ carpel type of construction. A transverse section of the 2-pistillate flower immediately after the emergence of the perianth cords shows a ring of vascular bundle masses, only six of which appear to contain any residual xylem (Fig. 162). At a higher level these masses unite to form a continuous ellipse in which the six groups of xylem are now very conspicuous. The bundles situated at the two ends of the ellipse later move out (Figs. 163, 164) and constitute the dorsal cords (sterile carpels), on the inner face of which the two loculi are formed. The two alternating vascular masses now divide in half between the two strands of xylem, one half of each mass pairing with the corresponding half of the opposite mass to form the ventral cord (fertile carpel) of the ovary on that side (Fig. 165).

Sorbaria (Figs. 180–2), *Sibiraea*. The various species examined, *Sorbaria sorbifolia*, A. Br. (*Spiraea Lindleyana*), *S. assurgens*, Rehd, *S. (Spiraea) Aitchisoni*, Hemsl., *Sibiraea laevigata*, L., all formerly included under *Spiraea*, differ from this last-named genus in regard to the position of the ovaries, which here, as in most Rosaceae, stand opposite the sepals without regard to the number of whorls in the androecium; also in having the stem apex convex. Hence we find, as we should expect, the characteristic arrangement of five dorsal cords (sterile carpels) on the radius of the sepals and on the alternate radii an inner whorl of five cords (fertile carpels) which divide in half, one half furnishing one of the two placental strands to the loculus on the right, the other a corresponding strand to the loculus on the left. This construction is precisely similar to that of the isomerous *Pomoideae*, each ovary being composed of $\frac{1}{2} \times \frac{1}{2}$ carpels. The same applies to *Gillenia trifoliata*, Moench., another genus closely allied to those cited above.

Pomoideae.

Amelanchier canadensis, Torr. and Gray (Figs. 178, 179). This genus is unique almost among the Rosaceae in having more or less bilocular ovaries.¹ These are composed, as in the allied genera *Pyrus* and *Cydonia*,² of $\frac{1}{2} \times \frac{1}{2}$ carpels, but in *Amelanchier* the sterile carpels are massive, and, although the ground tissue surrounding the dorsal cord leaves the central column of tissue, it is never entirely withdrawn to the ovary wall, consequently the ovary remains more or less bilocular.

¹ The only other case, apparently, is that of *Parinarium* (see p. 614),

² See New Phytologist, vol. xxiv, p. 206, 1925.

Cercocarpeae (Figs. 169-77).

Cercocarpus parvifolius, Nutt., and *C. betulaeifolius*, Nutt. (Figs. 169-75). The single ovary terminates the stem apex. After the emergence of the perianth cords (some eight in number although the corolla is wanting) a vascular ring is re-formed of bundle masses, most of which consist entirely of phloem (Figs. 169, 170). These masses gradually condense into the two xylem-containing cords (carpels) of the ovary. Though alike at first, one becomes fertile, the other remains sterile (Figs. 171-4). The single pistil thus resembles those of the *Sanguisorbeae* in consisting of two whole carpels, which, as in *Acaena*, may be indistinguishable in the early stages of development.

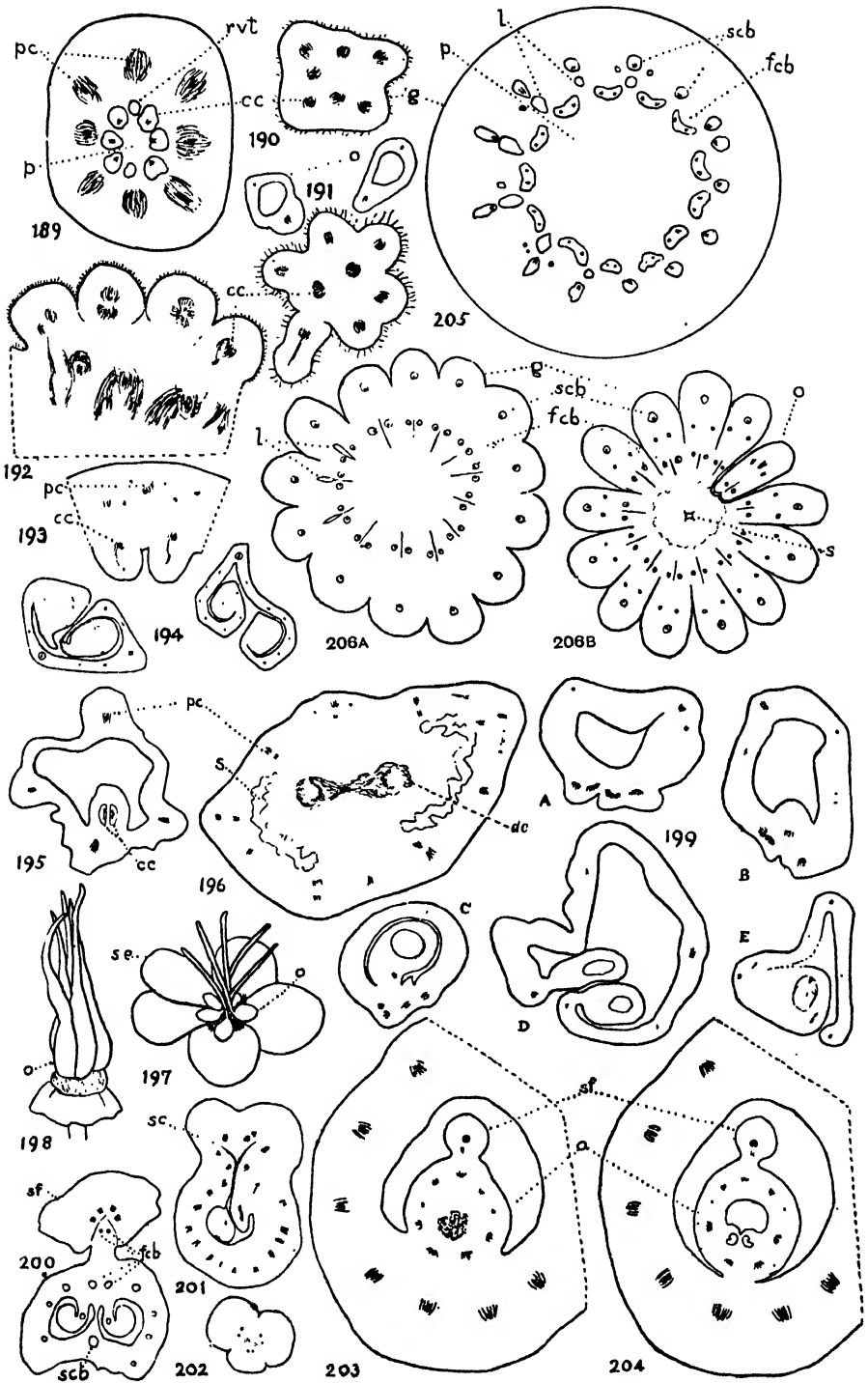
Purshia tridentata, DC. (Figs. 176, 177). Here also the ovary is solitary and terminal. Development follows much the same course as in *Cercocarpus*, except that in this case the two carpels can be distinguished from each other from the outset owing to their unequal development, the sterile member being expanded and forming wing bundles.

Quillajae (Figs. 183-8).

Exochorda Alberti, Reg. The 5-celled ovary becoming 5-winged in the fruit (Fig. 188) is almost superior, but otherwise resembles in structure the gynoeceium of the *Pomoideae*, and this is probably the case throughout this group. The sterile carpel cords leave the vascular cylinder conjoined with the sepal cords and separate later as the loculus makes its appearance (Figs. 185, 186). Extremely attenuated for so large an ovary, they cease altogether about half-way up, so that from this point a loculicidal split easily comes about. As development proceeds five deep furrows on the alternate radii extend from the outer surface inwards, almost up to the twin bundles of the inner fertile carpels, which are separated from each other by a small amount of ground tissue (Fig. 187). As the fruit ripens the intervening strip of tissue as well as the pith dries up, so that the five wings, each consisting of $\frac{1}{2}$ 1 $\frac{1}{2}$ carpels, readily break away.

Potentilleae (189-92).

This section differs from all those previously considered in that it is characterized by having numerous ovaries, though exceptionally the number may be much reduced, as in *Waldsteinia* (3-5), *Potentilla Tormentilla*, Neck. (sometimes as few as six or seven), species of *Ivesia*, *Sibbaldia*, *Cowania*, and *Potaninia mongolica*, Maxim, in which the ovary is solitary. The structure of the ovaries goes hand in hand with this retention (as we may presume it to be) of large numbers, for here for the first time we meet with the typical traditional apocarpous gynoeceium composed of monocarpellary pistils. The stem apex is usually convex or forms a cone-shaped mound in



the centre of a depression, on which the ovaries arise. This arrangement allows for the branching out from the axial vascular ring, after the emergence of the perianth cords, of further whorls of cords which supply the successive carpel whorls until the whole of the vascular tissue is thus used up, none of it being discarded as in most other sections of the family. Each gynoeical cord passes entire into a young ovary and there branches to form the dorsal and wing bundles, which latter here become, in reality, true marginal placentae. This mode of ovary formation has been observed in species of *Rubus*, *Fragraria*, *Potentilla*, *Geum*, and *Dryas*; also in *Waldsteinia geoides*, Wild., notwithstanding the reduction in carpel numbers in this species to three, or even two in the case of one of the specimens examined. In this latter case the young gynoeceum arose as a single protuberance projecting from the wall of the flower. The dorsal side of one ovary first became free, the line of separation from the flower tube then extended round one side of the gynoeceum to the dorsal line of the second ovary, and finally along the whole of the other side, thus leaving free a single structure which gave no hint in its outline at this stage that it would be fashioned into two ovaries. But in truth this structure represents more than the gynoeceum, for as the wing cords become defined a small vascular residuum—a last remnant of axial tissue which shortly disappears—is seen connecting the two ovary systems.

FIGS. 189–206. [All from transverse sections taken at successively higher levels, except 197, 198.] 189–91. *Potentilla Tormentilla*, Neck. 189 from flower A with six ovaries. The flower at the level of emergence of the perianth cords; the residual vascular tissue consists of eight masses, of which only six contain xylem. 190 from flower B with seven ovaries. The residual vascular tissue after emergence of the perianth cords is concentrated in seven xylem-containing bundles. 191 from about the same level in flower C, in which eight ovaries were formed; two are shown in section after they became detached. 192. *Dryas* sp. Part of one of the lower ovary whorls, behind which lies the vascular tissue destined for the next higher whorl. The single vascular cord of the monocarpellary ovaries becomes differentiated later into the dorsal and fertile wing bundles. 193. *Rosa setigera*, Michx. Part of the flower wall with two ovaries; the single vascular cord is branching to form the dorsal and wing bundles. 194. *Rosa indica*, var. *viridiflora* ('Green Rose'). Two leaf-like carpels with marginal ovules. 195. *Acaena ovalifolia*, Ruiz. and Pav. The perianth tube to which the single ovary is adherent; at this stage the two component carpels are indistinguishable. 196. *Spenceria ramalana*, Trim. The residual vascular tissue undergoes bifurcation previous to the formation of the bicarpellary ovaries. 197–9. *Kerria japonica*, DC. 197. Calyx and gynoeceum of the single flower. 198. Flower stalk with calyx base and gynoeceum from a double flower. 199. A–C from the lower, D, E from the upper portion of the gynoeceum of the double flower. In A–C the structure is similar to that of the ovary of the single flower, which is bicarpellary; in D and E the normally fertile carpel has come to an end, and the remaining carpel, which is ordinarily sterile, now takes on the ovule-bearing function, but the margins remain free. 200. *Parinarium macrophyllum*, Sabine. The gynoeceum showing below the loculus, partially divided, surrounded by the expanded sterile carpel and above the gynobasic style composed of two aborted carpels and part of the fertile carpel, the other portion of which projects into the loculus and bears the ovules. 201, 202. *P. capense*, Harv. 201. The same showing the 3-rayed stylar canal, one arm of the canal (that of the fertile carpel) being continuous with the loculus. 202. The style now disjoined from the ovary. 203, 204. *Chrysobalanus Icaco*, L. The gynoeceum attached to the perianth tube, a portion of which only is shown. In 203 the sterile carpel alone is differentiated, in 204 the twin bundles of the fertile carpel have taken shape. 205–6. *Eucryphia cordifolia*, Cav. 205. The gynoeceum showing an outer whorl of thirteen sterile cords (carpels) opposite some of which the loculus has already appeared, and an inner whorl of thirteen fertile cords (carpels) each with twin xylem strands. 206 A. The loculi are beginning to close in. 206 B. The individual ovaries are about to separate from the axial pith and to form the separate styles.

Roseae (Figs. 193, 194).

In the solitary genus included in this section, *Rosa*, the stem apex is generally deeply concave, the numerous ovaries arising in tiers from the wall of the concavity. Here, too, they are monocarpellary. This combination of a cup-shaped axis and numerous 1-carpelled ovaries is effected in the following way. The axis becomes considerably enlarged below the flower, and the whole vascular cylinder there breaks up to form a large number of cords arranged in concentric rings round the large area of pith. As the pith gives place to the central cavity the developing ovaries project inwards from the flower wall in successive whorls, instead of outwards from a diminishing cone of pith as in the *Potentilleae*. This regularity is beautifully seen in such a species as *R. moschata*, Mill. (*Brunonii*, Lindl.). As in the *Potentilleae* each ovary shows at its base a single cord which branches to form the dorsal cord and fertile marginal veins. In the so-called 'Green Rose' (*R. indica* var. *viridiflora*) the central leaf-like structures which replace the normal ovaries, although variously contorted and deformed by pressure, nevertheless bear marginal ovules (Fig. 194), affording strong corroboration of the monocarpellary nature of the functional way.

Chrysobalanoideae (Figs. 200-4).

This section comprises a group of genera characterized by the extreme basal position of the style filament, a construction which has always constituted a certain difficulty on the current view that the ovary is composed of one carpel.

Parinarium, *Acioa* (*Hirtellinae*) (Figs. 200-2). In these genera a deep tube on one side of the flower renders it markedly zygomorphic. The ovary, which is differentiated high up in the tube, and is adherent to one side, shows a most unusual type of structure. In *Parinarium macrophyllum*, Sabine, the gynoeceum has probably a ground-plan of six carpels: an outer whorl of three, of which two normally abort, one alone forming a loculus; and an inner whorl of three, of which one only is present and fertile.¹ The two aborted outer carpels, together with the midrib of the one fertile member, form a single massive style filament which separates from the ovule-bearing part of the ovary at its extreme base. Above, it bears a 3-lobed stigma. The loculus-forming carpel has a very well-developed vascular system (midrib and strong secondary veins). Opposite to it is the fertile carpel which thrusts its placental cords so far forward across the loculus that at the base of the ovary they are continuous with a projection of the wall on the opposite side, so that, at this level, the loculus is

¹ Juel has recognized this trimerous plan of construction, but taking only the valve carpel type into account he naturally conceives the whole gynoeceum as consisting of a single whorl. See *Arkiv för Botanik*, xiv, 7. p. 6, 1915; also G. Bonne, *Comp. Rend.* 182, pp. 1404-6, 1926.

divided. Its main cord (midrib) bends in the opposite direction, away from the loculus, to enter the gynobasic style which also receives the bundles of the two aborted outer carpels. On the radius of each set of bundles can be traced an arm of the stylar canal which is plainly 3-rayed. A similar structure was observed in another species, *P. capense*, Harv., and in *Acioa scandens*, Baill., though in the last-named form the ovary is scarcely bilocular.

A variation which has been recorded by different writers is the occurrence of more than one ovary in the flower. Baillon¹ states in regard to *Parinarium* that exceptionally we may find certain dicarpellary flowers produce two drupes. Again, D. Oliver² gives the number of carpels in *P. polyandrum*, Benth., as frequently two or more, and in *P. robustum*, Oliv., as one or three. In the absence of material of these species with more than one ovary it is hardly possible to pronounce a definite opinion as to what takes place in these circumstances. Possibly bi- (or tri-) partition of the vascular cylinder occurs, as has been shown to take place in numerous other Rosaceous types. Or it may be that the presence of two or three drupes signifies the full development of four or of all six carpels respectively.

Chrysobalanus (Chrysobalaninae) (Figs. 203, 204). Two species were examined, viz. *C. oblongifolius*, Michx., and *C. Icaco*, L. In this genus the flower forms a shallow cup. The single pistil is more centrally placed than in *Parinarium* and is adherent for a short distance only to one side of the flower wall. Only herbarium material was available of *C. oblongifolius*. Sections of this material showed a single loculus-forming carpel which, unlike its counterpart in *Parinarium*, becomes free from the flower tube before the style-forming members. The nature of the material made it difficult to determine with certainty the number of carpels contributing to the formation of the style filament. but so far as appeared the plan of construction is the same as in *Parinarium*. In *C. Icaco*, however, quite a different arrangement occurs, which, notwithstanding that excellently preserved material was available, is not easily interpreted. As the ovary protuberance becomes formed on one side of the flower wall the residual vascular tissue left after formation of the perianth cords is seen in transverse section as three bundle groups disposed round the pith, into which elements from these groups soon pass in considerable quantity. The two middle bundles of the unpaired group pass outwards into the style, where they coalesce to form a single strand. The other bundles of the group become ranged on either side as secondary veins, the whole group forming an arc round one side of the pith and later of the loculus. The two paired bundle masses condense into twin cords which spread out and complete the ring. The remaining vascular elements distributed in the pith become concentrated on

¹ Loc. cit., p. 424, foot-note 2.

² Fl. of Trop. Africa, ii, p. 378, 1871.

the radius, passing midway between the bundles of the paired groups (and hence opposite the style), and form the twin bundles of the fertile cord. It thus comes about that the style and the fertile cord are situated on opposite sides of the loculus, whereas in *Parinarium*, *Acioa*, and, as it seemed, in *C. oblongifolius*, the style and fertile cord stand in line on the same side of the loculus. The appearances described above suggest that the gynoeceum in *C. Icaco*, as in the three other cases cited above, consists of 3 + 1 carpels, but that in the former case the unpaired member of the outer whorl alone is used in the construction of the style, while all three go to form the wall of the loculus; whereas in the three latter types it is the two paired members of the outer whorl conjoined with the inner fertile carpel which together form the massive style filament, while the unpaired member of the outer whorl alone forms the wall of the loculus. If this interpretation is correct we have in *C. Icaco* the unique feature of the formation of a gynobasic style from a sterile carpel; in all other gynobasic-styled Rosaceae so far investigated it is formed from a fertile carpel.

Neuradoideae.

Examination of the structure of the gynoeceum of *Grielum humifusum* suggests a certain doubt as to whether this genus is rightly placed among the Rosaceae. Pending further investigation of this and the allied genus *Neurada*, discussion of this section has been deliberately omitted from the present account.

EUCRYPHIACEAE.

Eucryphia, the single genus included in this family, has a syncarpous ovary with distinct styles, equal in number to the loculi and, according to current views, also to the carpels, which are regarded as constituting a single whorl in which all are fertile. But in fact there occurs here, in a syncarpous, multicarpellary gynoeceum, a construction identical with that described above for certain Rosaceous types, viz. the 10-carpelled syncarpous ovary of *Exochorda* and the apocarpous pistils of the Pomoideae and of *Sorbaria*. That is to say, we have a 2-whorled arrangement with an equal number of outer sterile and inner fertile carpels, each loculus being enclosed by $\frac{1}{2} + \frac{1}{2}$ carpels (Figs. 205, 206 A). As the ovary merges into the style these $\frac{1}{2} + \frac{1}{2}$ combinations separate as so many unit structures (Fig. 206 B). In place of Gn we shall therefore write $Gn + n$, n varying in the genus from 51 to 8.

PAPAVERACEAE.

Eschscholzia californica, Cham. The accompanying scheme is intended to illustrate the progressive order of loss of styles and stigmas in this species, which has already been dealt with in considerable detail. It appears

on p. 134 of the earlier account,¹ but as, owing to reduction in size in reproduction, it does not there exhibit clearly the grading of vascular cords (as shown by size of the dots) it is here reproduced (Fig. 207). The explanation will be found in the earlier text.

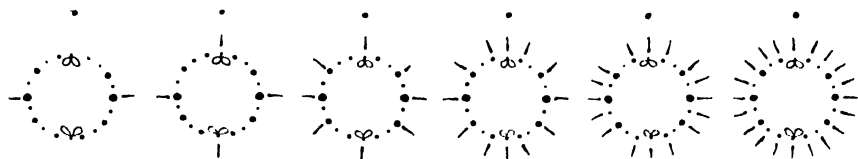


FIG. 207. Diagram to show order of loss of stigmas in *Eschscholzia californica*.

Hypercium procumbens, L. (Figs. 208–11). I suggested at an earlier stage of the inquiry² that *Hypercium procumbens* might, as regards its gynoeceum, represent a link between the many-carpelled types of the Papaveraceae and the typical Crucifer, and that the eight well-marked longitudinal ribs stood for so many consolidated carpels. A more complete investigation of the extreme base and apex of the ovary has shown that this suggestion is not borne out by further evidence.³ If traced to their origin in the short stipe of the ovary it is found that four out of the eight longitudinal cords arise as *lateral* veins, two from each lateral carpel midrib (Fig. 208). Further, that although these four veins run a separate course up the length of the ovary (Figs. 209, 210), they finally cease or reunite with the valve carpel midribs just below the stigma. The six bundles seen in a cross-section taken immediately below the stigma do not correspond to the similarly placed six sterile cords seen at a lower level, but to the midribs of the sterile and fertile carpels, the latter of which have split in two (Figs. 210, 211). It becomes clear from these facts that the *Hypercium* ovary is constructed on the same plan as the typical Crucifer silique, being composed of two sterile valve carpels and two solid fertile carpels.

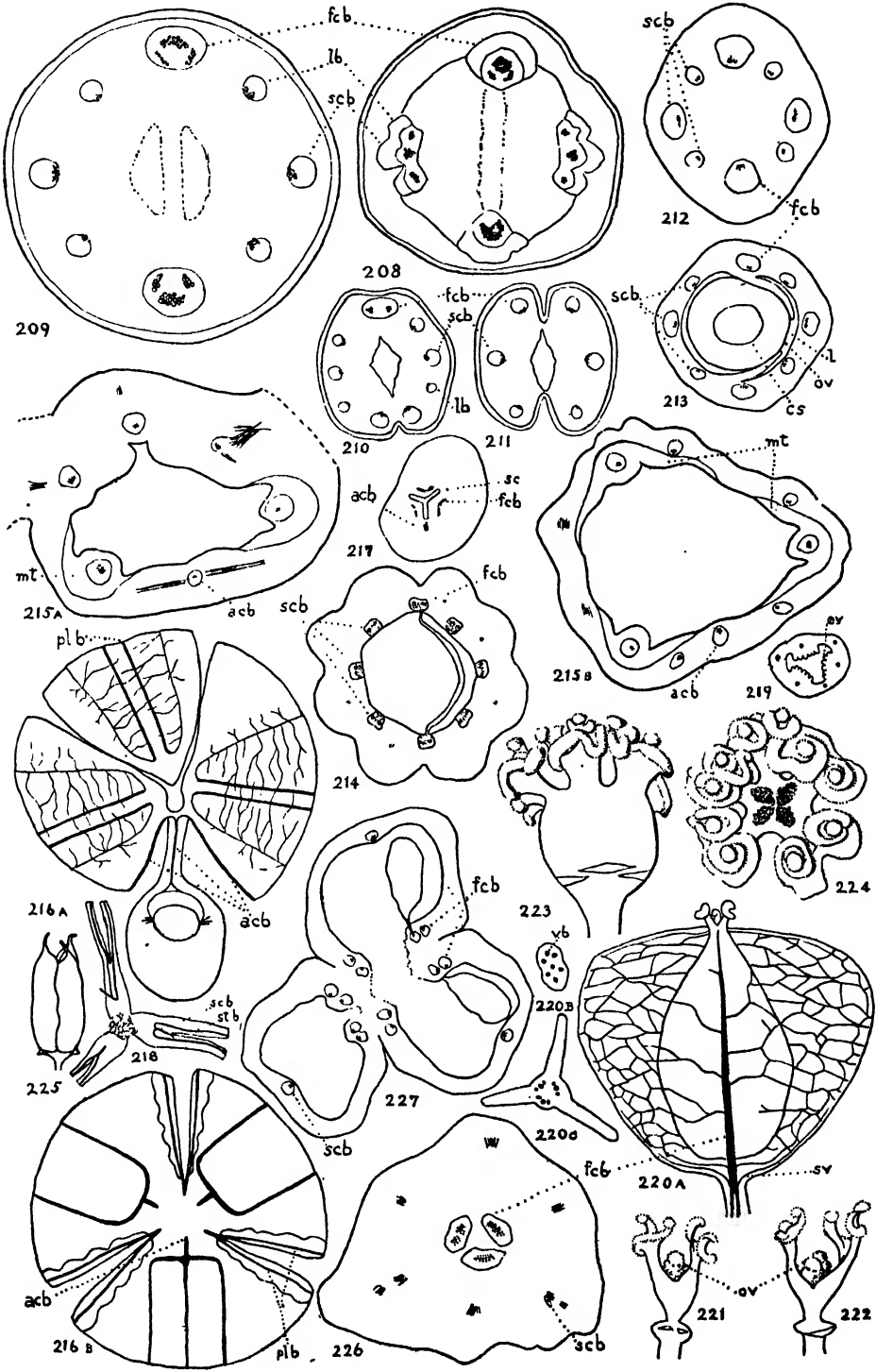
CRUCIFERAE.

Rapistrum rugosum, All. (Figs. 212–14). In contrast to *Hypercium*, in which the alternate longitudinal cords have been shown to be *lateral* veins, and hence not (as I had earlier supposed) indicative of carpel number, we may cite such a Cruciferous type as *Rapistrum*. In *Rapistrum rugosum* the four diagonal cords, when traced to their origin, are found, like the orthogonal four, to arise directly from the axial cylinder, and to persist to the top of the ovary. In this case there seems no doubt about the presence of two whorls, each of four consolidated carpels. Here the ridges conspicu-

¹ Ann. Bot., loc. cit.

² Ibid., vol. xxxvii, Fig. 58 and p. 479, 1923, and vol. xxxix, p. 144, 1925.

³ New Phytologist, vol. xxv, p. 299, 1926.



ous on the upper joint of the ovary are not, however, caused by the vascular cords, but are due to the bulging of the soft tissue between the cords.¹

These longitudinal bulges show no corresponding longitudinal bundle, though conceivably such bundles may have been present in some ancestral form. Were this the case the construction of the ovary would be comparable with that of the many-carpelled *Papaveraceae*.

R. Linnaeanum, Boiss. and Reut., although showing many more of these ridges, has also eight primary bundles and hence also but eight carpels. I now therefore think it probable, despite occasional records such as that of Schimper² for *Brassica oleracea*, in which he observed 10-valved pods, that my original estimate of a possible maximum for the family of 40–50 carpels is too high.

Matthiola incana, R. Br. It had been noted in an earlier account that in large cultures of the garden Stock young fruits are occasionally found split from above downwards for a larger or shorter distance in the median plane.³ As there pointed out this condition may result from the premature coming to an end of a fertile carpel, but examination of a considerable number of such fruits seems to show that it more often arises from an actual split of the fertile carpel itself in the mid-line between the twin vascular bundles. This kind of rupture, though an abnormal occurrence in *Matthiola*, is, as we know, a characteristic of solid carpels in many other families as the fruit ripens.

FIGS. 208–27. 208–11. *Hypocotyl procumbens*, L. The ovary cut across at the extreme base (208), at a somewhat higher level (209) and at the apex (210, 211). 212–14. *Rapistrum rugosum*, All. The ovary cut across at the base (212), through the lower joint (213), through the upper joint (214). 215–17. *Viola*. 215, 217. *V. Rydbergii*, Greene. The ovary cut across through the base (215 A), the middle (215 B), the apex (217). 216. *V. tricolor*, L. (semi-diagrammatic). A. The upper half of the ovary pressed flat, seen from inside; the style has been bent forward. The ovary wall has split, as in nature, along the mid-line of the three sterile carpels dividing in two the mid-rib of the anterior member, the two halves of which unite as they pass into the style. B. The lower half of the ovary split artificially in the mid-line of the fertile carpels, and pressed flat, seen from inside, showing the separate origin of the six carpel cords. 218. *Drosera capensis*, L. The ovary apex with the three forked style shanks. 219. *Passiflora caerulea*, L. The ovary cut across. 220. *Begonia Dregei*, Otto and Dietr. A. Young fruit with one wing and two placental cords removed, rendered transparent; in the centre one of the three fertile cords from which the system of secondary veins is derived. B. Flower stalk. C. Flower base. 221, 222. Two views of a male flower of tuberous *Begonia* from which the four perianth leaves and ten stamens have been removed, showing a superior ovary with partially exposed ovules. 223, 224. Two views of a female flower of tuberous *Begonia* from which the perianth leaves have been removed, showing a superior ovary with four whole styles and part of a fifth; the carpels are free above and the ovules exposed. 225–7. *Melanthium virginicum*, L. 225. Young fruit. 226. The flower base showing the six perianth cords, two out of the three outer sterile carpel cords (the third is not yet disjoined from the corresponding sepal cord) and the three inner fertile cords. 227. The three fertile carpels are tearing apart from one another in the centre and are splitting in two from the outside inwards; the fertile cords have also split in half and have given off the placental branches.

¹ In *Isatis*, another genus previously cited, the ridges, on the other hand, are due to veins. These, it has now been found, arise from the median carpel midribs; hence, as regards carpel number, they must now be held to be non-significant.

² See *Flora*, 1829.

³ *Ann. Bot.*, vol. xxxvii, p. 453, Fig. 9.

VIOLACEAE.

Viola, G 3 + 3 (hitherto regarded as G 3) (Figs. 215-17). A transverse section through the base of the ovary of Pansy or Violet shows six equidistant vascular cords (Fig. 215 A). Three of these cords—the midribs of three sterile carpels—give rise at once to a pair of strong lateral veins (Figs. 215 B, 216 B), from which spring the bulk of the smaller veins (Fig. 216 A); the other three belong to as many fertile carpels and furnish the placental cords. Of the three sterile carpel midribs the two posterolateral come to an end part way up the ovary, the anterior one alone persisting and supplying the style (Figs. 215 B, 217). The tissue lining the loculus becomes sclerosed as the fruit matures, except over the three sterile midribs (Fig. 215 B). Consequently, splitting takes place readily along these three radii. In the case of the anterior carpel the midrib becomes rent in two (Fig. 216 A), a rare occurrence in the valve type of carpel, if the sterile members are rightly regarded as such here.

DROSERACEAE.

Drosera rotundifolia, L., *D. binata*, Labill., *D. capensis*, L. (Fig. 218). The ovary of *Drosera*, like that of *Viola*, is unilocular, and in these species is composed of six carpels, three sterile alternating with three which are semi-solid and fertile. To which class of carpel the sterile members belong is not at once evident, as there is no secondary venation system to furnish a clue, but, as in the Violaceae, they are probably to be referred to the valve type. The styles stand over the sterile carpel midribs and, as Bugnon¹ has pointed out, are supplied by these midribs (which bifurcate on entering on the style shank) and not by the split fertile carpel cords, as had seemed to be indicated by Drude's figure in 'Pflanzenfamilien', III, 2, p. 262, Fig. 159, G, of which I made use in the earlier account.² This drawing, if intended, as I interpreted it, and as seems indeed to be beyond question, to illustrate the course of the carpel cords, does not appear on investigation to accord with the facts. But this discrepancy does not invalidate the evidence as to carpel number, as Bugnon, who denies carpel polymorphism *in toto*, would lead his readers to conclude. The true course of the vascular cords can be easily followed if the extreme apex of the ovary is sliced off and pressed flat as shown for *D. capensis* in Fig. 218, where the cords of the three sterile carpels which have torn free from the ovary wall, are seen to fork as they enter the style filament. The same disposition was observed in *D. binata* and *D. rotundifolia*, the latter being the species represented in

¹ La théorie du polymorphisme carpellaire et le cas des *Drosera*. Bull. Soc. Bot. de France, tom. lxxiii, p. 22, 1926.

² Ann. Bot., vol. xxxix, p. 143.

Drude's figure. Dehiscence of the fruit occurs, as in *Viola*, in the mid-line of the sterile carpels, rupture taking place sometimes alongside the midrib, sometimes by splitting it in two.

PASSIFLORACEAE.

Passiflora caerulea, L. (Fig. 219). The unilocular ovary, as in the two preceding families, shows three sterile and three fertile cords, the latter bearing numerous rows of ovules.

BEGONIACEAE.

The normal *Begonia* gynoecium has been discussed in the earlier account,¹ where I put forward the view that it is composed of three semi-solid carpels. This interpretation has been challenged by Bugnon,² who adheres strictly to the traditional conception and is wholly opposed to the idea of carpel polymorphism. Bugnon suggests that a small weak vein which runs from the centre outwards for a short distance near the lower margin of each wing and then ends blindly represents a carpel midrib, and that the gynoecium, as held hitherto, consists of three valve carpels of which these weak bundles are the midribs and the placental cords the marginal veins. That these weak veins are to be found in most, if not all, species of *Begonia* is quite true. They are shown in Fig. 220 A (*sv*) for *B. Dreyei*, Otto and Dietr., a white-flowered species which offers particularly favourable material for a study of the vascular system. But the mode of origin of these veins seems entirely to preclude the idea that they represent individual carpels, and certainly removes any possibility of regarding them as representing the only carpels present. The usual procedure in carpel formation is for a whole vascular unit or bundle to pass out from the axial cylinder, leaving a gap behind. These small veins do not arise as whole units, but as lateral branches of some of the seven main cords which are found in this species at the top of the flower stalk (Fig. 220 B), the branch sometimes being derived from the inner face of a cord and then curving round outwards. Another fact to be noted is that there is sometimes a distinct difference of level in the origin of these veins according with a similar difference in horizontal level of the wing bases, a feature not easily harmonized with the notion of a carpel whorl. Moreover, these veins do not appear to bear a definite relation to the seven axial cords, so that it is not possible to say with certainty from which cords or from which side of a given cord they will take their rise. Finally no symmetrical disposition of the central bundles is to be seen after these weak veins have branched off, such as one might have expected did these latter in fact represent the dorsal cords of a true carpel whorl. This lack of symmetry is, on the other hand, quite

¹ Ann. Bot., loc. cit., p. 151, and Fig. 59.

² Bulletin de la Société Linnéenne de Normandie, 7^e série, tom. ix, 1926.

comprehensible if these central bundles have not yet undergone final rearrangement prior to becoming leaf systems. When this process is completed the whole amount of vascular tissue is disposed in three bundle groups alternating with the wings, each group consisting of a central bundle (midrib) and two flanking bundles (Fig. 220 C), which later take up a central position and supply the placentae. The evidence for the presence of three semi-solid carpels could, indeed, scarcely be clearer, while if any lingering doubt as to the nature of the above-mentioned weak veins remained, it should, it seemed, be dispelled if one could meet with the rare condition in which the male flower produces an ovary which is then superior and, in consequence, without wings.¹ By chance I found during the present season among a number of tuberous *Begonias* a few flowers, both ♂ and ♀, with a superior ovary. Of the two ♂ specimens one had ten stamens surrounding an ovary closed below but open above, where the large masses of ovules were freely exposed (Figs. 221, 222). This ovary bore two normal styles, and on section proved to be bilocular, with the vascular arrangement characteristic of two semi-solid carpels. In addition, however, it showed on each side midway between the septa a central and two flanking bundles which might indicate two additional and sterile carpels alternating with the two fertile members, a possibility rendered still more probable by the different appearance of the tissue lining the loculus at this point. The second flower had no stamens; the ovary was trilocular with three normal styles, but otherwise showed precisely the same construction as the first specimen. But it at once became evident that no certain analogy could be drawn between these male flower ovaries and the normal gynoecium of the ♀ flower. For, in the first place, the ♂ flower ordinarily has numerous stamens, and hence the formation of a polymorous gynoecium as the result of the conversion of an unknown number of stamens into carpels would tell us nothing; secondly, even if the occurrence of alternate fertile and sterile carpels were established beyond question for these male flower ovaries, we have no grounds for supposing that the sterile members are the counterparts of the weak veins in the syngonous winged ovary of the female flower. It remained to examine the abnormal ♀ flowers. These bore in one case four complete styles and half of another (Figs. 223, 224), and in a second flower ten normal styles. The former of the two was sectioned. No bundles were found in the ovary wall between the septa which could be taken to stand for carpel midribs, the whole vascular supply consisting of

¹ These exceptional flowers, it may be remarked, furnish further proof that the ovary in the normal flower is not truly inferior. As I have suggested elsewhere (*New Phytologist*, vol. xxiv, p. 179) the normal condition here would be better described as *syngonous* instead of *epigynous*. In passing it may be noted that this well-known instance is not the only one in which disjunction of the organs serves to show the incorrectness of the current conception of the inferior ovary. This view has been put forward previously by Worsdell (*Principles of Teratology*, ii, p. 110), whose emphatic statement on this point was overlooked in my account of this type of ovary.

the midribs and placental bundles with their branches belonging to the semi-solid fertile carpels. We may then, I think, consider it to be definitely established that this weak vein in each wing of the syngonous ovary has no carpel significance. It forms part of the secondary venation system of the wings, which at this level are still solid. A second and third branch pass into the wings at successively higher levels (Fig. 220 A), but these structures having now split the vascular supply has to be duplicated, the bundles for one wall of the wing coming from the carpel on the right, the one for the other wall from that on the left. It is from these branches that the finer meshwork throughout the wings is derived.

LILIACEAE.

Melanthium virginicum, L. (Figs. 225, 227). I have previously shown that the Liliaceous gynoeceum usually consists of six carpels (three sterile and three fertile),¹ the ovary being generally trilocular with axile placentation and loculicidal dehiscence. In the genus *Melanthium* the ovary, however, becomes unilocular and has parietal placentation (Fig. 227). This distinction led me at an early stage of the inquiry to accept the orthodox explanation of a single whorl of 3-valve carpels for this section of the family.² A reinvestigation of *Melanthium* carried out upon more favourable material has shown, however (as I have since made clear³), that this interpretation is not borne out by the anatomical evidence, and that the ovary here, too, is composed of six carpels (Fig. 226). I may add that the orthodox interpretation of certain Crassulaceae also no longer seems to me tenable; even Ranunculaceous types (*Coptis*), previously cited, must be held doubtful until re-examined from the new standpoint.⁴ *Melanthium*, in fact, is one more case to be removed from the diminishing number of genuine instances of conjoined valve carpels with parietal placentation.

SUMMARY.

1. The current view that the unilocular ovary in Rosaceae is universally composed of a single carpel appears to be based largely on superficial appearance and ignores certain fundamental points of difference in its origin and construction in the various sections of the family.

2. A study of these differences has brought to light the existence of three main types of the one-chambered ovary, consisting of 1, $\frac{1}{2}$ 1 $\frac{1}{2}$, and 2 carpels respectively. A fourth type composed of more than 2 carpels occurs in a few species only.

3. The current (monocarpellary) view involves the serious difficulty that the supposed solitary carpellary leaf of many genera must be held to be terminal, as, for example, in *Prunus*, *Purshia*, *Cercocarpus*, *Margyri-*

¹ Ann. Bot., loc. cit., p. 158.

² Ibid., p. 126.

³ New Phytologist, vol. xxv, p. 304, 1926.

⁴ It is proposed to deal with these families in a later communication.

carpus, *Stephanandra*, *Chrysobalanus*, and those species of *Acaena*, *Alchemilla*, *Cliffortia*, and *Poterium* in which only one ovary is formed.

4. A further difficulty inherent in this interpretation in such a case as *Prunus* is the presence, at the level at which differentiation first becomes apparent, of a considerable arc of vascular tissue situated opposite the carpel midrib and between the two well-marked but widely separated 'wing' bundles, which are regarded on this view as fertile marginal veins. If the ovary were in reality composed of a single valve carpel the presence of this intervening arc would require explanation.

5. Neither the terminal position of the unilocular ovary nor the occurrence of this intervening arc of vascular tissue present any difficulty once it is realized that in the genera which exhibit these characters the ovary is composed of more than one carpel.

6. A certain real difficulty often encountered in the interpretation of gynoecia with from one to few ovaries arises from the fact that a great reduction in carpel number is often accompanied by two separate phenomena, viz. an asymmetric mode of development due to the attachment of the ovary to one side of the flower tube, and bi-, tri-, and even tetra-partition of the axial vascular cylinder preparatory to carpel formation.

7. In isomerous types the position of the outer whorl of carpels is not affected by variation in the number of whorls in the androecium. This constancy of position results from the fact that the stamen bundles pass out from the axial vascular cylinder conjoined with the perianth cords, only becoming independent at a much higher level. Hence the usual position of the outer carpel whorl opposite the sepals is in accord with the law of alternation.

8. Carpel formation appears to be dependent in large measure on the amount and position of the residual xylem after the perianth cords have left the axial cylinder. Phloem may be present in quantity, but if there is a lack of xylem it is discarded and no carpel is formed in this position.

9. Ovaries consisting of a single carpel are characteristic of genera in which large numbers are formed, as in *Potentilla*, *Fragraria*, *Geum*, *Dryas*, *Rubus*, *Rosa*. Individual species in these genera having the number of ovaries much reduced, such as *Potentilla Tormentilla*, Neck. (6-8), and *Rosa sericea*, Lindl. (sometimes not more than eight), nevertheless show the same type of construction. In genera coming under this head none of the vascular tissue is discarded, the whole of it being used in supplying the successive carpellary whorls.

10. In the above-mentioned monocarpellary types a single vascular cord enters the protuberance of the young ovary; a portion of this cord runs outwards to form the midrib, while the remainder gives rise to the two wing bundles, which here are truly marginal and ovule-bearing, and are generally prolonged above with the midrib into the style.

11. *Waldsteinia*, a genus included in the Potentilleae, which is exceptional in having the ovary number often reduced to three or even two, is also monocarpellary like its allies.

12. In most genera with separate ovaries equal in number to the members of a perianth whorl each is placed truly radially with the ventral face turned to the axis, and has the composition $\frac{1}{2} + \frac{1}{2}$ carpels. The whole carpel belongs to an outer whorl which usually stands opposite the sepals and is sterile; the two half carpels result from the splitting and divergence of the members of an inner fertile whorl in line with the petals. This type of construction is characteristic of the Pomoideae (*Amelanchier* alone is dealt with in the present account, *Pyrus* and *Cydonia* having been treated in an earlier discussion on perigyny), and of *Sorbaria*, *Sibiraea*, and *Gillenia trifoliata*. In the Pomoideae a certain amount of residual vascular tissue is discarded. The same construction is also met with in the oligomeric gynoceum of *Neillia*, in which only two or three ovaries are usually formed.

13. The $\frac{1}{2} + \frac{1}{2}$ formula also probably represents the ovary in *Rhodotypus*, *Kerria*, *Neviusia*, *Nuttallia*, *Spiraea* (in the strict sense), but in these genera the developmental process has been shortened by the elimination of an intermediate step. In *Rhodotypus*, where the stem apex is flattened, and in *Nuttallia* and *Spiraea*, where it is concave, this condensation is possibly attributable to the shortening of the axis which has diminished the area (height) available for the rearrangement of the vascular elements destined for the inner carpel whorl. If space restriction be the primary cause it must, however, be supposed to take effect in the horizontal rather than the vertical direction in *Kerria* and *Neviusia*, where the axis has remained convex. An indirect consequence of the concave form of the axis is that the radius of the outer carpels only coincides approximately with that of the perianth members (sepals in the case of *Nuttallia*, petals in *Spiraea*). The alternisepalous position of the outer carpels in *Rhodotypus* is due conceivably to the wide gaps separating the xylem elements in the residual vascular ring left by the exit of the sepal cords; in *Neviusia* it follows naturally upon the absence of a corolla; in *Spiraea* it remains unexplained. A considerable amount of vascular tissue is discarded in *Rhodotypus*, *Neviusia*, and *Kerria*, in which the stem apex is not concave.

14. In *Filipendula*, which has 5–15 ovaries disposed in a single whorl, each ovary is composed of two whole carpels, one sterile, the other fertile. The former is one of an outer whorl standing on one set of radii, the latter belongs to an inner whorl set on the alternate radii, hence the ovaries stand more or less askew. The curved course of the dorsal cord (sterile carpel) as it passes out from the vascular ring helps to counteract the deviation from a strict radial orientation which this type of construction would otherwise naturally involve. This tendency is further restricted in *F. Filipendula*, where the ovaries mostly range from ten to fifteen, by close packing.

But in *F. Ulmaria*, where the number is usually 5-8, this inclination sideways has fuller play and develops into a conspicuous twist.

15. Ovaries of two whole carpels are characteristic also of the Sanguisorbaceae (*Poterium*, *Acaena*, *Cliffortia*, *Spenceria*, *Margyricarpus*), and are probably universal in genera belonging to other sections where reduction in the gynoeceium has reached the limit, as in *Prunus*, *Purshia*, *Cercocarpus*, and *Stephanandra*. In *Prunus*, *Purshia*, and *Stephanandra* a larger or smaller quantity of vascular tissue is discarded.

16. The two genera *Agrimonia* and *Alchemilla* are exceptional in possessing an ovary destitute of a dorsal bundle, the fertile cord representing the whole of the vascular system. From analogy with other members of the family it would appear more rational to regard these ovaries as bicarpellary, in which the second carpel has become reduced to a non-vascular sac, than to consider the whole structure as composed of a single semi-solid carpel. Evidence on this point in the nature of proof is, however, at present lacking.

17. The solitary ovary of the Chrysobalanoideae, in which the style is completely gynobasic, exhibits a very unusual structure, consisting of an outer whorl of three sterile carpels and a single inner fertile carpel. In *Parinarium macrophyllum*, *P. capense*, *Acioa scandens*, and (?) *Chrysobalanus oblongifolius* two of the outer carpels abort and, united with the fertile carpel, form a massive style filament, the remaining outer carpel surrounding the locus. In *C. Icaco*, on the other hand, the unpaired outer member alone forms the style, while all the three members of the whorl are utilized to form the ovary wall. This latter species is unique in forming a gynobasic style from a sterile carpel.

18. The interpretation (see 15) of the normal *Prunus* gynoeceium receives additional confirmation from the fact that in some double-flowered forms (e. g. *P. avium*) the ovary is replaced by two small foliaceous leaves, or sometimes by only one leaf structure, in which case, however, the bundles of the second leaf can then be traced.

19. Supernumerary ovaries are frequently met with in the genus *Prunus* and arise in various ways. When two equivalent pistils develop directly from the stem apex, as occurs frequently in *P. lusitanica* and not rarely in the early blossoms of the Plum and Almond, they result from 'twinning' (bifurcation) of the vascular cylinder. Small additional ovaries are sometimes borne on the wall of the flower in the Plum, and here probably represent stamens transformed into single sterile carpels, since they show no ventral cord or ovules. In some double-flowered species the number may be further increased by the formation of secondary flower buds in the axils of the petals, as occurs in a double-flowered form of *P. triloba*.

20. The 10-carpelled gynoeceium of *Exochorda* appears as a 5-winged structure, each wing being composed of $\frac{1}{2}$ + $\frac{1}{2}$ carpels. The whole may be

compared with the gynoecium of the Pomoideae when freed from the axis, or with that of *Sorbaria* with ovaries coherent by their ventral face to a central pith throughout their length. G 5 + 5, splitting eventually into five portions, each of $\frac{1}{2} \pm \frac{1}{2}$ carpels, probably holds good throughout the Quilajaceae.

21. *Eucryphia*, the one genus in the small family Eucryphiaceae, has a syncarpous gynoecium of two isomeric carpel whorls with 5–18 carpels in each whorl, the outer being sterile, the inner fertile. The carpels of the inner whorl split in half, so that each loculus is bounded by one whole, sterile and two half, fertile carpels ($\frac{1}{2} \pm \frac{1}{2}$). This construction would be identical with that seen in the Pomoideae if the ovaries in the latter group were free from the surrounding axis and fused together below the styles.

22. It follows from the preceding statements that among the Rosaceae the *fertile valve* carpel occurs in those genera retaining a large number of ovaries, and also exceptionally in some closely allied genera having but few; but that where considerable reduction in the gynoecium is characteristic *consolidation* of the carpels is a general feature, and in these conditions the scheme of ovary construction varies from group to group, resulting in a structure of varying morphological equivalence, though constant, as a rule, within a genus. Or it may be put in this way: the *gynoecium* and the *carpel* are the constant elements—the fundamental units; the *ovary*, when not solitary, being a variable component of the larger and correspondingly variable combination of the smaller of these units which different genera are able to fashion differently out of the residual material. The three structures, gynoecium, ovary (when not identical with the gynoecium), carpel, exhibit in fact something of the relations of the molecule, the atom, and the electron.

23. The carpel number in certain genera belonging to other families has been found to be as follows: *Rapistrum rugosum*, *R. Linnacanthum* (Cruciferae), 4 + 4; *Viola*, *Drosera rotundifolia*, *D. binata*, *D. capensis*, *Passiflora*, 3 + 3. Reinvestigation has confirmed G 3 for *Begonia*; but it has become clear that the numbers 8 and 3 previously given for *Hypocoon* and *Melanthium* should be instead 4 and 3 + 3 respectively.

I wish here to tender my very grateful thanks to Miss D. F. M. Pertz for her kindness in making the drawings here reproduced, and to the Director of the Cambridge Botanic Garden for the free use which he has allowed me to make of the material available there. I desire also to express my gratitude to the Directors of Kew Gardens and of the Botanic Gardens at Kirstenbosch and Singapore, and to Professor McLean Thompson for the material received from them.

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A Cytological Study of Dormancy in the Seed of *Phaseolus vulgaris*.

BY

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With Plates XXVI and XXVII.

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INTRODUCTION.

WHILE engaged in a study of encystment in protozoa, the purpose of which was to determine, if possible, the significance of that phase of the protozoan life-cycle, the author became more broadly interested in the biology of 'latent life', and decided that a solution of this problem for one organism would be facilitated by a knowledge of other forms of 'resting' protoplasm.

It is a matter of common knowledge that many different plants and animals possess the ability to sustain life through periods of suspended activity during which the fundamental life processes are, to say the least, greatly curtailed. We find this latent phase appearing with great irregularity in the different groups. In some it always comes at a certain point

in the life-history. The encystment of *Cercaria* and the maturing of the seeds of higher plants furnish examples of this type where an embryonic form passes into latency. In some lower plants and some Protozoa spores or cysts respectively are formed in definite relation to other parts of the life-cycle, but here we have a single cell suspending animation and not a multicellular embryo, as mentioned above. In some Protozoa encystment appears to occur at any part of the life-history. This ability is not restricted to unicellular and embryonic forms, for, as we all know, many plants and cold-blooded animals can be frozen for months without detriment, and some animals (rotifers) can be dried for days in a desiccator and will revive when again supplied with water.

These facts, and their value to the species in tiding the organism over periods of adverse environment, are so obvious that most biologists are inclined to place a teleological interpretation upon them or to relegate them to the realm of those things whose survival value has made them practically universal, without attempting to discover what their actual value is. It seems to the writer that, at present, neither of the above positions is tenable.

It can readily be seen that latency may be intimately connected with some more fundamental problems of biology. In many cases it is closely related to fertilization in time, and it seems possible that it may be related in effect. There is strong evidence that encystment may be a means of rejuvenation in many Protozoa, and some of the rotifers that frequently undergo desiccation are able to continually reproduce by parthenogenesis, males never appearing. Thus it seems probable that desiccation makes amphimixis unnecessary. However, this whole field is practically untouched, and there is disagreement between some of the few studies that have been made.

As the subject may have bearing on one of the most fundamental problems of biology, rejuvenation, as well as many minor problems, it seems well worth consideration. The present cytological study was begun with a view to following the rule of making structure a basis for further study.

I wish to express my appreciation to Dr. C. H. Farr, Department of Botany, Washington University, for the criticism that he has very kindly given; to Mr. G. T. Kline, artist, St. Louis University School of Medicine, for his careful preparation of the plates; and to the Missouri Botanical Garden for the use of the library.

MATERIAL AND METHODS.

The seed of *Phaseolus vulgaris* was selected for this work. The variety commonly known as Stringless Green-pod Snap-bean was used.

The hypocotyl, plumule, radicle, and small pieces of the cotyledons

were the parts of the seeds used. Great numbers of these, belonging to five different stages of development, were fixed. The first stage consisted of beans that were very near full size, but which had not yet started to dry, the seed-coat still being a purplish-green colour. Those selected for the second stage were just beginning to dry, the seed-coat being a light brown colour. For the third step perfectly dry beans with a dark brown seed-coat were used. Beans that were partially permeated with water were used for the fourth, and germinated ones for the fifth.

All were fixed in Bouin's solution for twelve hours, slowly dehydrated, embedded in paraffin, and sectioned at three to six micra. Bouin's solution was used solely because past experience has shown that it is by far the best fixative when relatively large bodies are to be fixed. We have found corrosive-sublimate-acetic-alcohol and Flemming's strong very good fixatives for the peripheral layers of similarly sized tissue, but they are not sufficiently penetrating to fix all the way through.

With the exception of a few temporary mounts that were fixed and stained in aceto-carmine, iron-alum-haematoxylin was the only stain used. Iodine was employed to demonstrate starch.

HISTOLOGY OF THE SEED.

Immature Seed. The bean seed consists of two cotyledons which are attached to one end of the hypocotyl, and the plumule which grows out from the epicotyl between the two cotyledons.

The cortical layer of the hypocotyl consists of about eight layers of cells. The cells of the cortex are cubical and appear rather meristematic, though no mitoses have been observed, except in the tip. Inside the cortex comes a fairly thick layer of vascular tissue. The cells of this region are quite long and slender. The vascular layer extends into the epicotyl, forming an arch just below the proximate ends of the embryonic primary leaves. The pith forms a spindle-shaped mass within the vascular tissue. The cells of the pith are rather oblong, with their greatest dimension transverse to the long axis of the hypocotyl. The cells of the radicle are like those of the cortex.

The cells that make up the plumule are very small and there is little differentiation among them. There is a gradual increase of size as you pass from the plumule through the cortex of the epicotyl to the hypocotyl.

The largest cells of the bean seed are to be found in the cotyledons. Judging from the gigantic vacuoles seen in cleared sections, one would say that there is probably no more protoplasm in these cells than in those of the hypocotyl. A cotyledonary cell near the hypocotyl is not nearly so large as one farther out in the cotyledon. The vacuoles also are much smaller in the former.

In the cotyledons one finds reasonably good differentiation of the

epidermal layer. Some signs of this are seen in the plumule, but in the cortex of the epicotyl and the hypocotyl there is practically no epidermal differentiation.

There is nothing in the radicle end of the hypocotyl of the mature seed of *Phaseolus* to correspond to the root-cap which is later developed there. However, with the first few mitoses of the germinating seed, cells are formed at the distal end which cease dividing, and by the time the radicle is seen on the outside of the micropyle a well-developed root-cap is present.

The Dormant Seed. Minute structure of the cells will not be touched in this section, but only those facts which can be observed under a 16 num. objective. In the hardening process there is naturally a great amount of water lost. The visible effect of this loss of water is the same in this natural phenomenon as in plasmolysis of a cell in a hypertonic solution. This shrinking of the protoplasm, when complete, results in the protoplast filling only a small portion at the lumen of the cell. If the amount of shrinkage can be taken as an index of the amount of water previously in the cell, we can safely say that the medullary and vascular cells contain the greatest amount of water. The cortical and plumular cells and those of the radicle shrink somewhat more than the cotyledonary cells.

A significant feature of this drying is the fact that it does not occur as does the desiccation of inert bodies. The latter would dry first around the periphery, while in the hypocotyl of the bean the cells of the pith are the first to show signs of shrinkage, and this reaches an appreciable extent before the cells of the vascular area and cortex lose their turgidity at all. The cotyledonary cells follow those of the pith, and the cells of the cortex, radicle, and vascular area and the plumule are the last to shrink.

The fact that the pith shows evidence of drying first proves quite conclusively that the desiccation is not a passive one, but that there is some mechanism within the seed which actively throws off, or draws off the water that is within it. Otherwise, drying would begin on the outside. Since such a condition is found only at the beginning of desiccation, one can safely say that it is not due to fixation (Pl. XXVI, Figs. 1-6).

Distribution of Starch in the Growing and Dormant Seed. In the unripe seed one finds many large starch grains in the cells of the cotyledons. This same condition continues after desiccation, with the exception that the grains stain more darkly with iodine than those of the growing cotyledon (Pl. XXVI, Figs. 1 and 4). The cells of the cortex and pith of the hypocotyl of unripe seeds are well filled with very small starch granules. The granules are so small and evenly distributed that, with the low power of the microscope, the cells when stained with iodine appear almost as a homogeneous blue mass (Pl. XXVI, Figs. 2 and 3). Occasionally this same condition is found in some of the plumule cells bordering the main

veins. In the dry mature hypocotyl the above condition is materially altered. The few plumule cells that contain starch and the medullary cells show much fewer granules, and they are larger than in the growing seed, although not nearly so large as the grains in the cotyledons (Pl. XXVI, Fig. 5). The greatest change is seen in the cortical cells, which in unripe seeds are like the pith as to starch content. In dry seeds there is ordinarily no starch whatever in these cells. Occasionally a cell is found containing a single starch grain, but in practically all of them the starch is either utilized or transported before drying (Pl. XXVI, Fig. 6). The distribution of starch in the cells of the radicle is the same as in the cortex. In no case was any starch found in the cells of the vascular tissue.

The germinated bean shows identically the same distribution of starch as do seeds before ripening.

THE PREDORMANT CELL.

In the growing embryo of an unripe seed the cells in all of the different parts are quite evenly turgid, the cytoplasm completely filling the space bounded by the cell-walls. Fixed and stained plumule and hypocotyl cells, in thin sections, show the cytoplasm to consist of slender strands of coagulated material scattered irregularly in the cell, thus leading one to believe that, in the living condition, these cells contain a high percentage of water (Pl. XXVII, Fig. 7). The cells of the cotyledons, on the other hand, with the exception of the starch grains, appear to contain a much greater abundance of coagulated cytoplasm (Pl. XXVII, Fig. 19). In the cotyledonary cells one finds numerous small basophilic granules in the cytoplasm. These occur less commonly in the embryo. They may be metachromatic granules, but no conclusion was reached as to their exact nature.

In the resting nucleus of the cells of such an immature bean, one of the most prominent features is the eccentric chromatin nucleolus or karyosome. Surrounding the karyosome is a rather wide perinucleolar zone which is hyaline except for the linin strands running from the karyosome to the nuclear membrane. In many cases the disperse chromatin obscures the distal end of these strands. The space from the outer edge of the perinucleolar zone to the nuclear membrane is filled with relatively large chromatin granules suspended on a none too definite network (Pl. XXVII, Fig. 7). The chromatin granules are much larger and less numerous in the cells of the plumule, hypocotyl, and radicle than in those of the cotyledons, where they are not only smaller and more numerous, but are also arranged with much greater regularity (Pl. XXVII, Fig. 19).

THE DORMANT CELL.

As stated above, the most striking change coincident with the approach of desiccation is the shrinkage of the protoplasm. The cytoplasm of dormant

cells shows evidence of much more definite organization than that of growing cells, having within it extremely regular starch grains which give it, in cleared sections, a foam-like appearance. The rest of this account will not deal with this fact, or to any great extent with the cytoplasmic condition during dormancy, but will be practically confined to observations on the nucleus (Pl. XXVII, Figs. 9 and 13).

With the shrinking of the cytoplasm is a similar decrease in the size of the nucleus, and its outline is irregular. The regular outline is again observed after desiccation is complete. Also one can observe that the chromatin granules are gradually becoming smaller and more numerous and that the perinucleolar zone is being invaded by particles of chromatin. This process continues until the granules are very minute and very numerous and the perinucleolar zone has disappeared (Pl. XXVII, Fig. 8). Sometimes the granules are almost entirely lacking in an area half-way between the karyosome and nuclear membrane.

Meanwhile the karyosome has been increasing in size, and the linin strands connecting it with the nuclear membrane appear to become heavier and stain more darkly.

By the time the seed has become completely dry the nucleus contains a very large karyosome. The space between this body and the nuclear membrane is hyaline (Pl. XXVII, Figs. 9 and 13). The greatly increased size of the karyosome alone would indicate that a large part of the disperse chromatin has entered that body. In addition to this the perinucleolar zone has been invaded by particles. This would point to the same conclusion. The karyosome of dormant cells frequently is not spherical as it was before desiccation (Pl. XXVII, Fig. 18).

In most of the sections there did not appear to be any chromatin around the periphery of the nucleus (Pl. XXVII, Figs. 9 and 13). However, in overstained sections one can see a thin layer of chromatin pasted around the inside of the nuclear membrane (Pl. XXVII, Fig. 17). Though it cannot be definitely stated, it is our personal belief that this thin layer of chromatin is always present, but is rather easily destained. Temporary mounts fixed and stained in aceto-carmin showed this layer of chromatin around the nuclear membrane.

The basophilic granules in the cytoplasm of cotyledonary cells, mentioned above, are still present, and in addition the cells of the embryo are fairly filled with similar bodies. The increase in the number of these chromatic granules of the cytoplasm is concomitant with the shrinking of the protoplast. No chromatin was seen passing from the nucleus. Many of the sections were greatly destained in order to avoid the complication introduced by these cytoplasmic granules, and this probably accounts for the observations noted in the last paragraph.

The ultimate results of drying are the same in all parts of the embryo

(cf. Pl. XXVII, Figs. 9, 13, and 20). The drawings are principally taken from the pith, but cells from the radicle or any other part of the seed could have been used equally as well.

THE POSTDORMANT CELL.

When dormant beans are placed in a germinator one notices great lack of uniformity in the rate at which the various seeds absorb water and great variation in the length of time before the inception of growth. Sections of seeds that have been in a germinator only a short time show that the cells of the plumule, radicle, and cortex of the hypocotyl are the first to become turgid, while those of the pith and cotyledons are the last.

The nucleus returns to the normal active condition by passing through the same steps, in reverse order, that were passed through during the hardening of the seed. The nucleus enlarges, and very fine granules of chromatin begin to appear in the region surrounding the karyosome, while the latter body shows a decrease in size. The chromatin granules again become larger and fewer, and we have reached the condition which prevails in the nuclei of an immature bean. The irregularity of the outline of the nucleus during the changes is again seen (Pl. XXVII, Figs. 10, 11, 14, and 21).

DISCUSSION.

Before discussing this topic it is necessary that we review the few related observations that have been made by previous workers. Most of the work that has been done on seeds is of a physiological nature, or has dealt with general morphology, not cytological details. Köppen (10), in an investigation of the nuclei in the cells of dormant seeds, noted that the outline of the nucleus in starch-bearing cells of dormant seeds was very irregular. The irregularity disappeared during germination. It is interesting that this author came to the conclusion that some of the nutritive cells of the seed do not contain a nucleus. Peters (13) observed that in germinating seeds the size of the nucleus increases. The nucleolus also swells, and in some cases 'accessory nucleoli' appear.

The only thorough cytological study of the changes resulting from desiccation is that of Hickernell (6). This author found that, in rotifers, during drying the nucleolus disappears and the chromatin becomes evenly distributed over the inner surface of the nuclear membrane. If animals are fixed while dry this material stains very lightly, but when they are placed in water for a short time previous to fixation the chromatin, though still on the nuclear membrane, has regained its affinity for dyes. By the time the animal has become active the chromatin is again in the nucleolus. At the same time an incomplete study was made of the corn grain. The observations here indicated that a similar change occurred.

In addition to the study of normal cells in the dormant condition, several workers have carefully investigated the structure of cells under experimental conditions. Zacharias (22) discovered that the nucleolus of *Spirogyra* decreases in size when kept in the dark. Schiller (15), using *Antithamnion*, confirmed this observation.

Schrammen (16), in a study of the effect of temperature on cells, found that at 0° to 5° C. the nucleus is irregular in outline, and the nucleolus and disperse chromatin granules increase considerably in size. Around 40° C. the nucleolus shrinks, and sometimes it entirely disappears. The chromosomes of dividing cells kept at this temperature are inclined to clump. Hartmann (5), in a very convincing and beautifully illustrated paper, entirely confirmed the above observations of Schrammen. Hovasse (7) found that when frogs' eggs are permitted to develop at 0° to 4° C., the chromosomes are incompletely alveolized during the telophase, so that fragments of them persist. Division is normal at 13° C.

Němec (12) noted an enlargement of the nucleolus as a result of plasmolysis.

To summarize this experimental work: the nucleolus enlarges under plasmolysis and at low temperatures, while it becomes smaller at high temperatures and in the dark. The enlargement of the nucleolus in these instances may be attributable to the same factor as its enlargement during dormancy in the bean, namely, the migration of a part of the scattered chromatin into the nucleolus.

The differential drying of the regions of the hypocotyl is of especial interest. What mechanism makes it possible for the pith to shrink before the cortical and vascular cells have lost an appreciable amount of water? It would seem that the cytoplasm of the various cells must contract or lose its ability to hold water within itself, thus producing shrinkage first in the inner cells. Many will probably say that such a suggestion is 'far-fetched', but, after all, it is no harder to conceive such a mechanism here than it is to assign a non-passive role to the protoplasm which transforms a chromosomal vesicle into a chromosome or a spermatid nucleus into the compact head of a spermatozoon. A rather plausible physical explanation of this seems to be found in the fact that starch is present in both pith, cortex, and radicle in the full-sized, undried bean, while it is found only in the pith after ripening. If this material is transported from the cortex and radicle to some other part of the seed just previous to desiccation it would entail the presence of an appreciable amount of sugar in this area, while such crystalloids may not be present in the pith. Now, when the supply of water from the plant is cut off we would expect those cells containing sugar (cortex and radicle) to remain turgid at the expense of those containing starch (pith).

The large intracellular spaces in the dry seed must be an important factor in the early absorption of water by seeds. The presence of air in

such spaces would probably explain the great lack of uniformity in the rate of germination of different seed of the same variety, and also the fact that absorption of water by a single seed is not perfectly regular throughout its germination. In case this air should become blocked off in certain areas it would have to go entirely into solution before the water could permeate that area.

The perinucleolar zone seems generally to be regarded as an artifact due to fixation (Sharp (17); De Smet (3)). The fact that, with the same fixation, it is found only during certain phases of the development of the seed of *Phaseolus* might be interpreted to mean that it is not an artifact. The evidence, however, is not sufficient for such a conclusion, in the face of previous work, and the subject must await further observations. If it is an artifact we must conclude that the nucleolus of partially dry seeds is more firmly attached to the nuclear reticulum than the nucleolus of unripe or germinated seeds.

The cytological response to desiccation in the rotifers differs greatly in detail from the changes herein described for *Phaseolus*. However, there seems to be a fundamental similarity in that, in both cases, the chromatin is in an extremely compact form during the dormant period. We are not justified in assuming that there is any physiological relationship between dormancy of seeds and desiccation of rotifers.

At present our knowledge is not sufficient for us to pronounce with any degree of certainty the exact significance of dormancy of seeds. Increased resistance to external conditions is doubtless a part of it, but what part we do not know. It might be suggested that the nuclear condition during dormancy represents a mere extension of the resting phase. This, however, does not seem probable since, in both plants and animals, tissue cells which have divided for the last time do not lose their distributed chromatin or in any way resemble the dormant cells of *Phaseolus*, and we would be inclined to think that such cells which will never divide again have reached the ultimate resting condition.

It is barely possible that dormancy of seeds is one of the steps in rejuvenation. The strong probability that dormancy is a rejuvenating process in Protozoa and rotifers makes it worth while for us to examine the few bits of evidence which might mean that it is a similar phenomenon in plants. In the first place, is there any similarity between the nuclei of the cells of a maturing seed and the nuclei of unquestionably senescent cells? Lutman (11) has made a very complete study of the cytological changes accompanying senescence in the potato plant. He notes that in old cells the stainable material of the nucleus continually diminishes and the nucleolus shrinks until it almost reaches the point of disappearance. In rejuvenating cells he finds an increase of chromatin. The same thing is beautifully illustrated by fatigued or senescent nerve-cells. As a result of

fatigue, or a gradual wearing out in old individuals, the Nissl bodies and the chromatin of the nucleus disappear. If these are entirely used up the cell cannot regenerate, but when the cell can do so the chromatic material is replaced. It should require no argument to show that there is no similarity to be found here, and no convincing support for the theory that dormancy of seeds is a period of rejuvenation.

Amphimixis is generally considered to be a rejuvenating process and, as mentioned in the introduction, there is in many forms a definite time relation between this and dormancy. This is not true for parthenogenetic rotifers, cold-blooded animals (those that can be frozen), or certain phases of the life of some plants. In this seasonal adaptation dormancy does not come at any definite point in the life-cycle.

In most animals fertilization initiates a period of growth which does not cease until the individual has become an adult. Fertilization in the sporozoa and some thallophytes stimulates the formation of a protective covering for the zygote and a varying period of dormancy. In mosses and ferns fertilization is followed by growth and the dormant spores are produced as the end-point in the life-cycle. In higher plants amphimixis is followed by a period of growth and food storage, and dormancy comes while the individual is still an embryo.

The great difference in the outcome of fertilization in higher plants as compared to most animals is significant, and reminds us of the few things that the gametes of the two have in common. In some ways the spermatozoon and the egg of animals are more comparable to the plant seed than to the gametes which give rise to it. The compact condition of chromatin in the sperm nucleus and the quantity of stored yolk in the egg are matters of common knowledge. In the gametes of the higher plants an entirely different condition prevails. The male nucleus is of the ordinary resting type, and the female cell is not filled with food, but is receiving its nourishment from the sporophyte. In fact, so far as these two points are concerned the mature seed is more similar to the animal gametes. The condition of the chromatin is very much like the head of a spermatozoon, as shown in the present work, and the stored food (starch) in the cytoplasm is comparable to the yolk in the egg cell. If this structural similarity between plant seeds and animal gametes is of any significance we must consider fertilization in seed-bearing plants as a premature phenomenon, occurring before the perfect elasticity of sex-cell rejuvenation has completely rebounded, and dormancy of seeds as the final step in this process. This is given merely as a possibility.

Is there any experimental evidence to show that this is true? The illuminating research from the Hull Botanical Laboratory has shown that the impermeability of the seed-coat to oxygen and water is usually responsible for delayed germination. However, the seeds of some plants must

pass through an after-ripening period before they will germinate, even with the seed-coat removed. Sherman (18) observed that, in some seeds, catalase and oxidase content increases for several months during the after-ripening period. The above facts indicate that if dormancy should prove to be a rejuvenating process, in most seeds it occurs during the ripening period and not during the after-ripening period.

Jacobs (8) and Hickernell (6) suggest that desiccation of rotifers makes amphimixis unnecessary. The former, in a series of carefully conducted experiments, found that a high rate of reproduction followed desiccation.

SUMMARY.

1. In the drying of a bean the cells of the pith are the first to show shrinkage. Eventually all of the cells in the seed shrink appreciably.

2. In the growing bean chromatic granules are found in the cytoplasm of cotyledon cells, and more infrequently in the cells of the embryo. In dry beans they are very generally found throughout the entire seed.

3. A great number of very fine starch granules are found in the cortex and pith of the hypocotyl and the radicle of developing seeds. In the mature seed a few are found in the pith, none in the cortex or radicle.

4. In the drying of the seed the scattered chromatin granules either enter the karyosome or become evenly distributed over the inner surface of the nuclear membrane, thus leaving the space between karyosome and nuclear membrane hyaline.

5. On germination the nucleus returns to the normal condition.

6. The nuclear changes are probably quite similar to the effect of low temperature and of plasmolysis on nuclei.

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EXPLANATION OF PLATES XXVI AND XXVII.

Illustrating Dr. J. M. Kater's paper on a Cytological Study of Dormancy in the Seed of *Phaseolus vulgaris*.

Figs. 1-6 are photomicrographs of sections six micra thick, stained with iodine. Figs. 7-21 made from sections four micra thick, stained with iron-alum-haematoxylin. All material fixed in Bouin's solution. All drawings made with Abbe camera lucida. Magnification, Figs. 10-24, $\times 2,100$.

PLATE XXVI.

Photomicrographs.

Fig. 1. Section through the cotyledon of a growing bean. Note large starch granules with evident hila.

Fig. 2. Section through pith of hypocotyl of growing seed. The many minute starch granules give a rather homogeneous appearance.

Fig. 3. Picture showing cortex and vascular tissue. The distribution of starch in the cortex is identical with Fig. 2; none in vascular area.

Fig. 4. Section of the cotyledon of dry seed.

Fig. 5. Section through pith of hypocotyl of dry seed. Note the presence of a few small starch granules.

Fig. 6. Section of cortex of dry seed. No starch present.

PLATE XXVII.

Fig. 7. A cell from the pith of immature bean seed. Typical resting cell.

Fig. 8. Cell from pith of a seed just beginning to dry. Note slight shrinkage of protoplast and chromatin granules entering the karyosome.

Fig. 9. Cell from the pith of hypocotyl of dormant seed. Typical appearance during dormancy.

Fig. 10. Cell from pith of hypocotyl of a seed lately placed in germinator.

Fig. 11. Cell from pith of hypocotyl of a germinated seed.

Fig. 12. Cell from plumule of an unripe seed.

Fig. 13. Cell from plumule of a dormant seed.

Fig. 14. Cell from plumule of a seed that has just begun to grow, after dormancy.

Figs. 15 and 16. Cells from the pith and cortex respectively of the hypocotyl of a seed shortly after being placed in a germinator. Note that the nucleus of cortex cell has more nearly approached the condition of Figs. 10, 14, and 15.

Fig. 17. Cell from the pith of the hypocotyl of a dormant seed. Note layer of chromatin around the inside of nuclear membrane.

Fig. 18. Cell from the pith of the hypocotyl of a dormant seed. Note irregular form of karyosome.

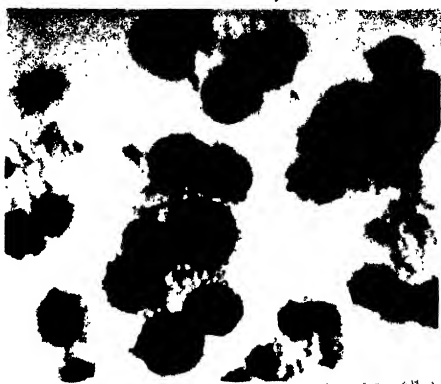
Fig. 19. Cell from the cotyledon of an unripe seed.

Fig. 20. From the cotyledon of a dormant seed.

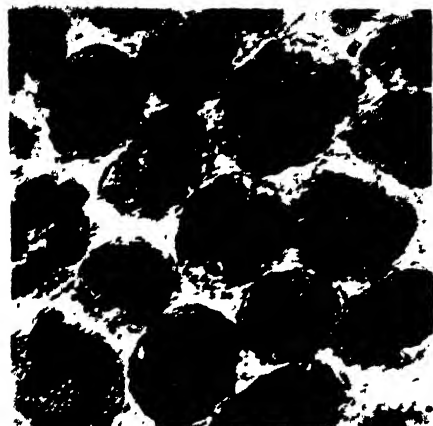
Fig. 21. Cotyledonary cell from a germinated seed.



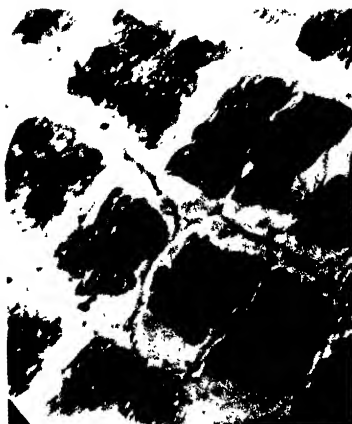
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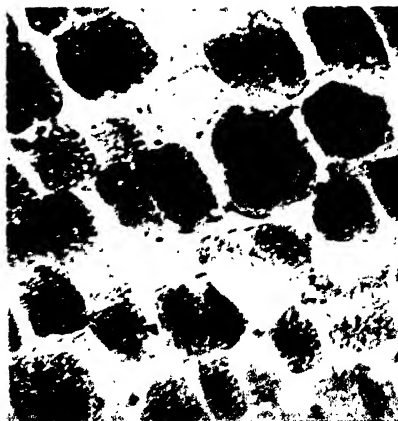
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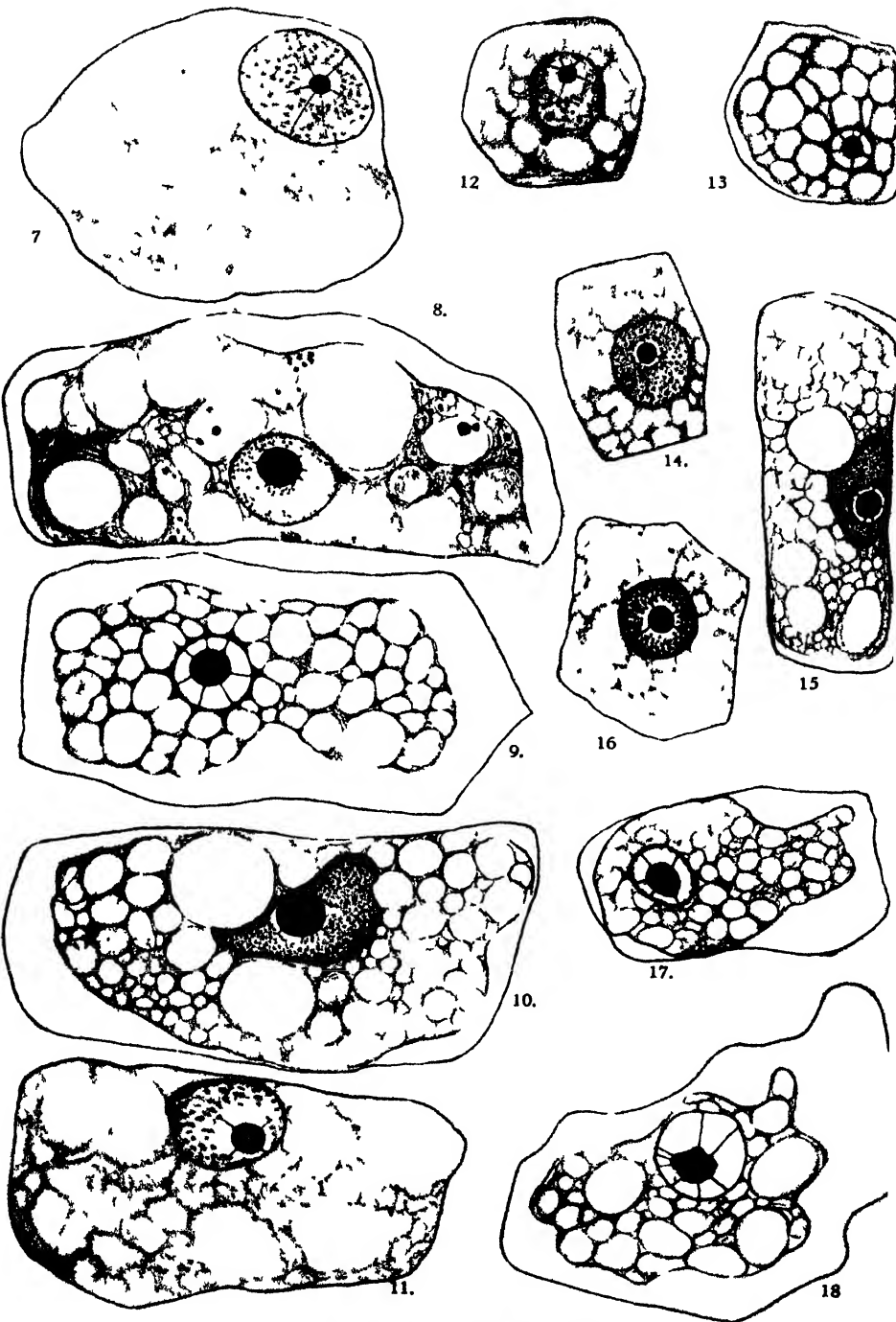
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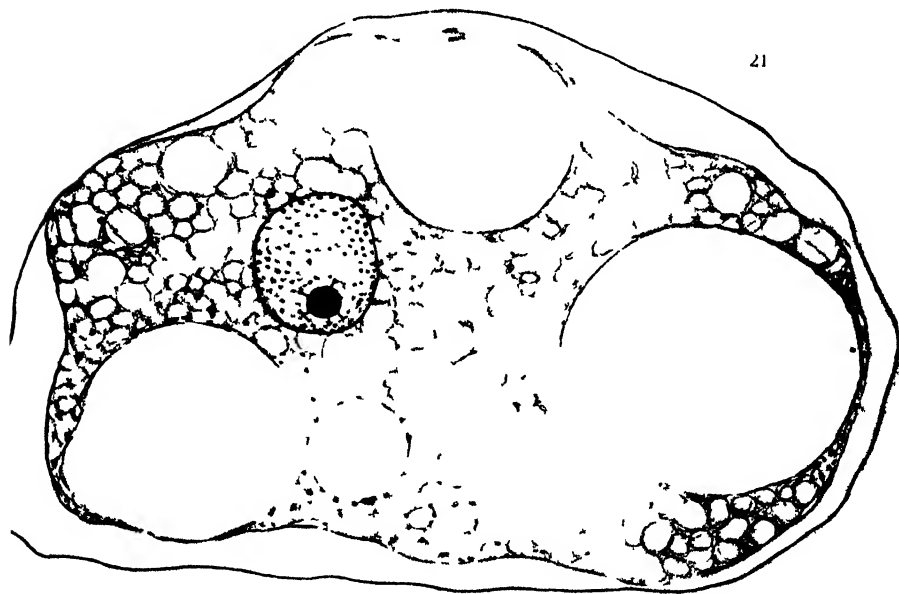
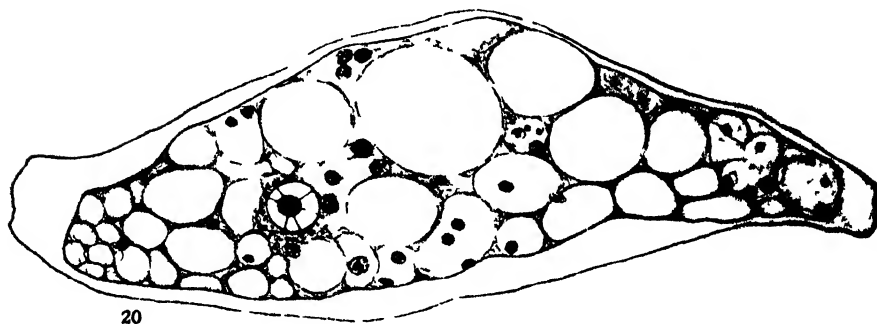
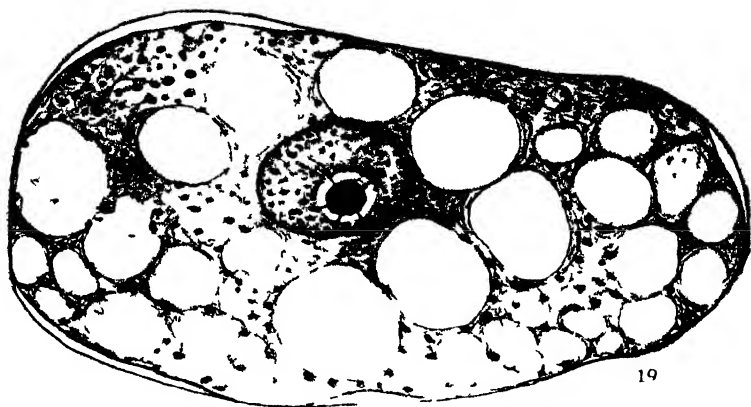
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KATER — SEED OF PHASEOLUS.

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KATER—SEED OF PHASEOLUS.



Studies in the Physiology of Parasitism.

X. On the Entrance of Parasitic Fungi into the Host Plant.

B 7

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AND

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With one Figure in the Text.

IN an earlier paper dealing with the physiology of parasitism (3), a brief sketch was given of certain lines of research which were then in progress and which were concerned with the mechanism of the entry of the parasitic fungus into the host tissue. More recently these investigations have been carried forward to a further stage, so that a full statement of the results is now rendered possible.

The problem of the mode of entry of such a fungus as *Botrytis cinerea*, which penetrates directly into the uninjured epidermis, involves two distinct subsidiary problems, (a) that of the nature of the stimulus which induces entry, and (b) that of the means by which the fungus is able to overcome the resistance of the plant cuticle. In the former connexion the view which is most commonly put forward is that the stimulus is of a chemical nature (chemotropism), though it has also been suggested that contact stimulation (stereotropism) may play a part. As regards the means possessed by the fungus of penetrating the cuticular barrier, there are also two alternative theories. According to one view the fungus penetrates by means of some cuticle-dissolving enzyme; according to the other, by mechanical action alone. In the present paper, certain experimental results will be put forward which have a bearing on the above questions. For the sake of clearness the problem will be discussed under the headings of the two subsidiary problems already mentioned.

A. NATURE OF THE STIMULUS INDUCING PENETRATION.

The view that chemotropic factors play a dominant part in stimulating fungal penetration is due mainly to Miyoshi (8, 9), who examined the response of fungal germ-tubes to various chemical substances along the

lines laid down previously by Pfeffer in his studies of bacteria and other motile organisms. The experiments of Miyoshi which are most apposite to the present discussion are those dealing with membrane penetration. The membranes tested comprised collodion films, parchment, gold leaf, cellulose film impregnated with paraffin, together with natural membranes such as the epidermis of *Allium* scales. According to Miyoshi various fungi could be induced to penetrate these membranes, but only when a certain concentration of some attractive chemical substance was placed on the opposite side. Similarly in the case of living leaves of various plants (*Tradescantia*, *Begonia*), it was claimed that while no entrance of the parasite took place when the leaf was intact or merely injected with water, active penetration resulted when the leaf was injected with a solution of a suitable chemical, e.g. cane sugar. From these and similar experiments Miyoshi therefore concludes that chemotropic factors are fundamental in determining the penetration of fungal germ-tubes.

Miyoshi's work is to a large extent undermined by the later work of Fulton (5). The latter, using the same methods and a similar range of fungi, was unable to confirm many of Miyoshi's results. He states in fact that the fungal hyphae show as much turning towards pure water as towards a solution of any particular chemical, and that the dominant directive stimulus is one which causes the fungus to grow out of a medium where there is an abundance of fungal hyphae to a medium from which hyphae are absent. He thus postulates in place of Miyoshi's positive chemotropism a negative chemotropism of the fungal hyphae to their own staling products.

Finally Graves (6) reinvestigated the problem and arrived at conclusions which afford a compromise between Miyoshi's and Fulton's views. Graves was able to show that the negative chemotropism of Fulton is the major effect, but that a positive chemotropic effect of various chemical substances also exists, though not to the marked degree claimed by Miyoshi. Graves further found that the positive chemotropic effect of a natural decoction, such as turnip extract, was much greater than that brought about by cane sugar alone, and suggests that natural plant juices are more chemotropically active than any of the simple chemical substances hitherto experimented with. The identity of the particularly active substance or substances present in turnip extract was not determined.

Graves's work represents the latest important contribution to the subject of chemotropism. It now remains to examine how far the two chemotropic factors established by Graves are of service in explaining the initial penetration of fungal parasites.

In considering the negative chemotropic or 'staling' effect as a possible cause of fungal penetration, one must point out in the first place that there is a considerable difference in degree between the amount of the staling factor brought into play in Graves's experiments and the amount of staling

which may take place in the infection drop. To obtain his negatively chemotropic medium, Graves used a turnip extract in which *Rhizopus nigricans* had grown for three weeks. There was thus a considerable quantity of fungal material growing for a considerable time. Now in the case, for example, of the attack of a bean leaf by *Botrytis cinerea*, a single spore lying in the infection drop is sufficient to initiate attack, and that within twenty-four hours of the placing of the drop on the leaf. The amount of staling material produced in the latter case must be negligible. Thus Graves's experiments are hardly relevant to the case of penetration. Apart from this consideration, however, it appears to the present writers that any attempt to explain penetration on the basis of negative chemotropism to staling products is not sound from the purely physical point of view. This may be seen by a consideration of what must be the mechanism of the tropic process. The latter can only arise when a different concentration of the active chemical substance is maintained on opposite sides of the growing hypha. In the case of the negative tropism under consideration, a higher concentration of the 'staling' products on one side will lead to turning of the hypha away from that side. When a hypha is growing in free liquid, the rate of outward diffusion of staling products will be uniform round the growing zone, and therefore there will be no turning; if now the rate of diffusion is slowed down on one side of the growing hypha, as for example by placing the latter on a relatively impermeable surface such as a plant cuticle, the effect would be to raise the concentration of staling substances on the side of the hypha next to the cuticle. According to this theory, therefore, the hypha should grow away from and not towards and through the plant epidermis.

The alternative chemotropic view, that fungal germ-tubes can appreciate the unilateral outward diffusion of certain chemicals from the host tissue, and react to this diffusion gradient by turning against it, is at least feasible from the physical point of view. While it is difficult to disprove the existence of such a tropic factor, it has been possible to show that penetration of a series of artificial and natural membranes can be brought about under conditions where this tropic factor is ruled out. The following is an account of such experiments.

(a) *Experiments with Paraffin Membranes.*

Miyoshi's general statement is that fungi only penetrate membranes when a chemotropic influence of some particular substance on the opposite side of the membrane comes into play. Nevertheless, he himself showed that fungal germ-tubes were able to penetrate a membrane composed of cellulose impregnated with paraffin wax (9, p. 276). In view of the high degree of impermeability of paraffin wax, this result does not agree with the chemotropic theory, and it is clear from Miyoshi's own description that

he found this result difficult to explain. This result, in fact, if it could be substantiated, would definitely dispose of the theory that a chemotropic stimulus arising from the opposite side of a membrane was necessary for the penetration of the latter.

In repeating these experiments it was decided to replace the paraffin-impregnated cellulose membranes of Miyoshi by membranes of pure paraffin wax only, in order to get rid of the possible objection that some solution of the cellulose by the fungal enzymes might take place. These paraffin wax membranes were cut from a paraffin block 1 by 1.5 cm. in cross-section, in the usual manner, with an ordinary Cambridge rocking microtome. The paraffin used varied from 45° to 52° M.P., and sections were cut at intervals of 5 μ from 5 μ to 30 μ in thickness. Each individual section was then tested for impermeability in the following manner. It was first of all placed on a few drops of indicator solution (brom-thymol blue, which was just acidified with hydrochloric acid so as to have in bulk a pale straw colour) on a glass slide which was then slightly warmed in order to make the sections lie perfectly flat. The slide was then put in a moist Petri dish, a drop of 1 per cent. caustic soda solution laid on the centre of the wax membrane, and the whole placed in an incubator at 20° C.

When the preparations were examined on the following day it was found that in the great majority of cases no change of indicator colour had taken place, so that the paraffin membranes were impermeable even to such a simple electrolyte as sodium hydroxide.¹ In those preparations in which the colour of the indicator had been changed to blue, it was clear that the portion of the membrane in contact with the drop of alkali contained a flaw. In nearly every such case it was seen that the drop of alkali touched one of the fine striae which here and there cross the membrane and which are due to irregularities in the edge of the microtome knife. This trouble can be avoided, or at least reduced to a minimum, so that it is not difficult to prepare section after section which will prove to be impermeable when tested as above described.

In no case was the impermeability of the membrane taken for granted, but each was tested separately. Furthermore, it was found possible to test fungal penetration over exactly the same area of the membrane as had been previously tested for impermeability. When the drop of caustic soda was removed by touching it with a pipette, it was seen to have produced a slight indentation of the membrane. This spot was then washed with several successive drops of water, and the drop containing the fungal spores

¹ There appear to be some grounds for believing that paraffin wax is slightly permeable to water. Thus it is claimed that when material of a starchy nature is embedded in paraffin wax it is very difficult to cut, whereas when the paraffin blocks are kept for some time in water the difficulty is much lessened. This suggests the slow inward diffusion of water resulting in the softening of the starch.

was finally placed on the same spot. It was thus possible to guarantee that the part of the membrane in contact with the spore-containing drop was impermeable to one of the simplest electrolytes, and therefore, it must be presumed, to any of the substances the chemotropic effect of which was under consideration.

For the actual penetration tests spores of *Botrytis cinerea* were sown in water or in various concentrations of turnip extract. The membrane was placed on a layer of plain agar or of turnip extract agar on a slide. From the point of view of penetration of the paraffin membrane it is obvious that the composition of the medium underlying the membrane can be of no significance, since the latter is quite impermeable to solutes. However, all the possible arrangements of nutrient in relation to position of spores were tested, partly for the sake of completeness, and partly because by this means the accidental presence of spores beneath the membrane could be checked. The preparations were then incubated at 20° C. Penetration was determined by inverting the slide and examining under the low power. There was no difficulty in deciding which hyphae were below the membrane and which above. Determinations were made simpler still by removing the liquid from the spore drop, replacing by a drop of ruthenium red, and allowing ten minutes for staining. The hyphae on the original upper surface were then strongly stained, while those on the other side of the membrane were unstained.

It was found that penetration took place eventually with all the paraffins used and for all thicknesses tested provided the spores were sown in nutrient. In no case was penetration obtained from a suspension in pure water, though small appressoria were occasionally found on the surface of the wax. With sections of 5–10 μ of 45° M.P. paraffin, penetration was seen after twenty-four hours. With increasing thicknesses of membrane and with harder wax, the time varied from forty-eight hours to three days. Sections of 45° M.P. wax of about 1.5 mm. in thickness were penetrated in five days by spores sown in dilute turnip extract. In this case fresh nutrient was added from time to time to the spore-containing drop.

It may be noted here that while the presence of nutrient below the membrane has no effect on penetration itself, it renders penetration more obvious when it does take place. In such a case the hyphae which have penetrated grow more vigorously beneath the membrane, branch, and form attachment organs on the glass, so that their presence there is rendered more evident.

The above experiments therefore completely confirm Miyoshi's observations and show that, in this case at any rate, penetration has taken place in the absence of any positive chemotropic factor.

(b) *Experiments with Membranes of Formalized Gelatin.*

The experiments with these membranes are of greater interest in connexion with the mechanical theory of membrane penetration and will therefore be described more fully later.

It may be pointed out that these membranes, even when they are so hard that the fungus is unable to penetrate them, are highly permeable to crystalloids. Provided the membrane used is not too hard, hyphae of *Botrytis cinerea* and other fungi penetrate if sufficient nutrient is available. The tests were set up according to the following schemes:

- (1) Spores sown in turnip extract ; membrane imbibed with water.
- (2) " " water ; " " " turnip extract
- (3) " " turnip extract ; " " " " "

Penetration took place in all cases, and was thus independent of the initial distribution of the nutrient. The sole requirement is that nutrient should be available to the spore to allow of vigorous growth. When one bears in mind the rapid rate of diffusion of crystalloids as compared with the rate of spore germination, it is difficult to believe that any concentration gradient which might be present at the beginning of the experiment could last long enough to influence the direction of growth of the young hyphae. Further, the experiment of type (1) clearly shows that penetration has taken place in spite of any chemotropic influence which may have been produced as a result of the initial distribution of the nutrient.

(c) *Experiments with Natural Membranes.*

The upper epidermis of *Allium* bulb scales, being free from stomata and being easily removable, is a suitable object for experiment. Miyoshi showed that *Botrytis* spores readily penetrated this membrane, the presumption being that they were induced to do so by the attraction of some substance diffusing out from the injured epidermal cells. This experiment has been repeated with the like result. In order to test whether any chemotropic substances present in the epidermal cells played any part in the process, the epidermal strips were subjected to a thorough washing in water (to which a trace of chloroform had been added), with a view to removing this diffusible substance. Penetration of the washed membranes took place with the same ease as before. Again it was found that the fungus penetrated as readily from the inside to the outside of the membrane as vice versa, thus proving that the penetration of the cuticular and outer epidermal layer could not have been brought about by the presence of any chemotropic substance in the epidermal cells.

A similar series of tests was carried out with epidermal preparations of *Eucharis Mastersi* and *E. amazonica*. The epidermis in both these species is free of stomata. The method of preparing the membranes is as

follows. The leaf is laid, upper side down, on a smooth surface, preferably on a sheet of paper, and the underlying tissue is removed by careful scraping along the direction of the veins until only the transparent upper surface of the leaf remains. These membranes are then tested for the presence of holes, either originally present or resulting from the process of preparation, by placing a solution of Congo red dye on the outer surface for some hours. The presence of a hole is shown by a small stained spot, caused by the dye coming in contact with the epidermal cells, whereas the cuticle remains quite unstained.

Membranes of *Eucharis* prepared in this way gave results identical with those described for *Allium*.

The above experiments, both with artificial and natural membranes, show conclusively that membrane penetration takes place freely under conditions where chemotropic influences are completely excluded.

B. METHOD OF CUTICULAR PENETRATION, MECHANICAL OR CHEMICAL.

Later in the present paper a discussion will be given of what appear to be the relative merits of the mechanical and the chemical theories of cuticular penetration. In the meantime an account will be given of some experiments which add to the already available evidence that fungi do possess the power of penetrating membranes by purely mechanical means.

It may be remarked first of all that Miyoshi's experiments with gold-leaf, collodion, and paraffined cellulose, the latter of which in a modified form have been fully confirmed by the present writers, establish the fact that fungi possess the power of penetrating membranes in a purely mechanical way.

(a) *Experiments with Membranes of Formalized Gelatin of Graded Hardness.*

The method of preparing the membranes has been described elsewhere by one of us (4), and briefly is as follows. Strips of air-dried gelatin about 1 in. by 1 in. are cut from ordinary sheet gelatin, or preferably from a sheet of gelatin obtained by drying down a 5 per cent. gelatin solution which has been poured over a mercury surface. By standardization of details it is possible in the latter way to ensure that all the strips used are of the same thickness. The strips are next subjected to a process of differential imbibition, by placing them in a series of alcohol-water mixtures (0, 20, 40, 60, 80 per cent., &c., alcohol) for about twenty-four hours. A given quantity (1 c.c. to each 10 c.c. of alcohol-water mixture) of 40 per cent. formaldehyde is then added. After twenty-four hours' treatment in the formalin-water-

alcohol mixtures, the membranes are transferred to water and thoroughly washed to remove all the traces of alcohol and formaldehyde. The addition of formalin to the membranes while the latter are still in the alcohol-water mixtures is for the purpose of destroying as far as possible the capacity of gelatin to absorb further water, so that when the membranes are subsequently transferred to water the graded effect still persists. Grading is shown by the fact that the membranes are progressively less swollen, are harder, and have a higher gelatin content (lower water content) as the series of alcohols is ascended. The following series of figures, quoted from the paper mentioned above, illustrates the graded effect in terms of the gelatin content of the membranes.

0 % (i. e. water).	20 % alc.	40 % alc.	60 % alc.	80 % alc.
7.9 %	13.0	24.7	34.7	42.5

These membranes are fairly stable in watery solutions (apart from acids and alkalis), and can be used to illustrate the penetrating power of fungi. Their method of preparation ensures their sterility in the first instance, and by the use of sterile water for washing, &c., contamination with foreign organisms can be avoided.

The method of carrying out the experiments was the same as described earlier in the case of the paraffin-wax membranes. The spores were sown on the upper surface in a good nutrient, such as turnip extract; the imbibition liquid of the membranes was either water or the same nutrient in which the spores were sown. The dishes (moist Petri dishes) containing the preparations were incubated at 20° C. as usual.

In each experiment the results are usually shown clearly on the second day from sowing. The slides are examined under the low power from the under side, and no trouble is experienced in deciding when penetration has taken place. Apart from difference of level, the penetrating hyphae show a characteristic zigzag or even corkscrew habit of growth as contrasted with the normal appearance of hyphae growing on the surface. The following is a tabular account of a particular experiment. The graded series of membranes was prepared by use of 0 %, 10 %, 20 %, 30 %, 40 %, 50 %, 60 % alcohol solutions. The amount of penetration, as estimated after two days' growth of the fungi, is given in the following table:

Membrane :—	0 %.	10 %.	20 %.	30 %.	40 %.	50 %.	60 %.
<i>Botrytis cinerea</i>	strong	strong	strong	fair	little	none	none
<i>Penicillium glaucum</i>	fair	fair	fair	little	none	none	none
<i>Rhizopus nigricans</i>	strong	fair	little	none	none	none	none
<i>Mucor</i> sp.	strong	fair	little	none	none	none	none

In the above experiment, which was carried out in triplicate, with complete agreement between the three sets, it will be noticed that *Botrytis* penetrates the 40 per cent. membrane, whereas penetration by *Rhizopus* is stopped by

the softer 30 per cent. membrane. From the point of view of penetrating capacity this experiment shows that *Botrytis* exceeds *Penicillium*, which again exceeds *Rhizopus* in penetrating power. Perhaps the comparison between *Botrytis* and *Penicillium* is not fair to the latter, as some allowance should be made for its slower rate of growth, but it is clear from the table above that *Rhizopus* possesses a smaller penetrating capacity than *Penicillium* in spite of the greater growth-rate of the former. The limits of penetration for the first three fungi, as given in the above table, are :

<i>Botrytis cinerea</i>	40-50 per cent.
<i>Penicillium glaucum</i>	30-40 " "
<i>Rhizopus nigricans</i>	20-30 " "

No attempt has been made to determine more accurately the membrane of limiting hardness for each case, chiefly because the actual figure has no absolute value. The hardness of any particular membrane depends on the degree of swelling that it has undergone, which, as is well known, depends on a great variety of factors, including the nature of the particular samples of gelatin used. The figures, therefore, are only relative, and any comparison of the penetrative capacity of different fungi should be made with membranes prepared, preferably simultaneously, from sheets of the *same* sample of gelatin.

The question remains as to whether penetration of these gelatin membranes is brought about by chemical or by mechanical means. While it is well known that many fungi attack and liquefy ordinary gelatin, there does not seem to be any evidence that formalized gelatin is similarly attacked. In the present work no trace of such action was observed. The characteristic zigzag growth of the hyphae within the membrane substance strongly suggests mechanical penetration. A similar type of growth is described and figured by Miyoshi when hyphae are penetrating collodion membranes, and is likewise interpreted by him as indicating mechanical penetration.

That penetration is due to mechanical and not to chemical action is also indicated by the following considerations. As shown in the above table, the hyphae of *Botrytis cinerea* had penetrated the 40 per cent. membrane, but not the 50 per cent. one. This was after growth for two days. On prolonging the experiment for some days longer, no further change took place, i.e. the 50 per cent. membrane still proved impenetrable. The renewal of the food supply to the fungus also made no difference. The fungus, in fact, grew on the surface of the 50 per cent. membrane, as if the latter were composed of glass—that is, there was abundant formation of appressoria, but no penetration took place. If now the fungus penetrated in virtue of possessing some gelatin-softening enzyme, it is difficult to see why the 50 per cent. alcohol membrane should not also be penetrated in

course of time. Both membranes, the one which is penetrable and the one which is not, contain in the neighbourhood of 70 per cent. of water in the form of imbibition liquid, so that if enzymatic action is possible in the one case, it should be so in the other. The fact, therefore, that the 50 per cent. membrane remains unpenetrated throughout indicates that the factor determining penetration is the mechanical hardness of the membrane, and that penetration by the fungus takes place by mechanical means.

(b) *Experiments with Leaves of Eucharis Mastersi and E. amazonica.*

It has been already stated (p. 649) that the detached upper epidermis of either of these two species of *Eucharis* is readily penetrated by *Botrytis cinerea*. Nevertheless, when spores are sown in nutrient on a piece of the living leaf, no penetration takes place even after a period of five days' incubation, by which time the leaf is beginning to turn yellow. A very striking way of carrying out this experiment is as follows: A piece of leaf is taken and a small epidermal 'window' is prepared in the centre by scraping away the tissue beneath the epidermis after the manner already described. The 'window' is then backed with any of the following: water, or a variety of nutrient solutions, or the expressed sap of *Eucharis* leaf petiole, with or without the addition of agar in each case. Spores of *Botrytis cinerea* in nutrient (turnip extract) are then sown on the upper side of the 'window', and also on the surrounding parts of the leaf. After twenty-four to thirty-six hours' incubation at 20°, strong penetration has taken place from the drop placed on the 'window'. On the other hand, no infection takes place after several days from the drops laid on the living portions of the leaf, even when fresh nutrient is added from time to time to the drops in order to prevent the incidence of staling conditions. A considerable mass of mycelium with appressoria is produced, but when this is rubbed off the underlying tissue appears green and unaffected,¹ and microscopic examination shows that the latter is free of fungal mycelium.

Hyphae which have penetrated the edge of 'windows' in this experiment spread freely in the surrounding leaf tissue, and produce a very obvious killing and rotting effect. Similarly, when a small piece of epidermis is removed and spores are laid on the cut surface, rapid attack takes place from the wound outwards. *Eucharis* leaf tissue is therefore quite susceptible to *Botrytis* attack under these experimental conditions, so that failure to produce attack when the spores are laid on the intact living leaf is to be set down to some property of the cuticle. Apparently also the resistance

¹ From time to time a number of small reddish spots were observed on the surface on which the spore-drop had lain. The same also appeared, and with similar frequency, under control drops of nutrient from which the spores were omitted. The nature of these discolorations was not made out, beyond the facts that they were not caused by the fungus and that their presence was not associated with any fungal attack.

of the cuticle to penetration is reduced by removing the living tissue from behind it.

Healthy pieces of *Eucharis* leaf were hung in an atmosphere of chloroform vapour for twenty-four hours, after which the chloroform was removed under reduced pressure. These killed leaves were found to be readily penetrated by *Botrytis*, the leaf tissue becoming rotted and filled with hyphae within forty-eight hours after the sowing of the spores. Portions of the leaf which were kept alive as controls remained unaffected as usual on inoculation. The same result was obtained with leaves killed by exposure to sulphur dioxide or formalin vapours, by soaking in alcohol, by dipping in boiling water, and by freezing at 23° C., and also with leaves which had died naturally on the plant. Again, leaves of *Hedera helix*, *Nerium Oleander*, and *Prunus Laurocerasus*, all of which are devoid of stomata on the upper surface, showed exactly the same behaviour as has been already described in the case of *Eucharis*.

The results described thus appear to possess some degree of generality. Similar results have been reported by Brooks (1) for lettuce, and by Pfaff (12) for *Echeveria*, *Phyllocactus*, and *Ficus*, though in both these cases the possibility of entrance through the stomata was not excluded. It is a well-known fact that moribund tissues are more readily attacked by *Botrytis cinerea* and such fungi than are healthy parts, but it has generally been assumed that this difference is due to the greater permeability of the unhealthy tissue, in consequence of which more food material leaches out into the infection drop. In the present experiments abundant food provision was made for fungal growth in all cases, so that the results obtained are to be ascribed to some change in the resistance of the cuticle itself.

The decreased resistance to penetration shown by the cuticle of killed leaves could not be due to a greater degree of imbibition of the cuticle (and consequent softening) resulting from the liberation of sap from the killed cells. Both in the case of killed and living leaves, the portion of cuticle which is under test is in contact with the drop of liquid containing the fungal spores. Each has therefore a similar opportunity to imbibe liquid up to saturation point, so that one cannot postulate a difference arising in the manner suggested.

As another possible factor, one may consider the injection of the intercellular spaces which always takes place in the case of the killed leaves. Miyoshi (9) and Massee (7) claimed that leaves which were injected with nutrient (chemotropic) solutions were penetrated by fungi which do not attack the normal leaf. Both these writers, however, describe the germ-tubes as entering through stomata, so that their results do not bear directly on the present problem.

To test the effect of injection of the intercellular spaces, leaves of *Eucharis* were injected with one or other of the following liquids: water,

dilute turnip extract ($\frac{1}{10}$ strength), 1 per cent. glucose, various synthetic nutrient solutions, including Richards's and a glucose-asparagin-mineral salts medium.¹ On testing these injected leaves with spores of *Botrytis cinerea* in the usual way, no penetration was found to have taken place after three days' incubation at 20°. It thus appears that injection of the intercellular spaces, even with a complete nutrient, is not in itself the cause of the diminished resistance to penetration.

There remain two other alternatives, that the diminished resistance to penetration in the killed leaves is due either (1) to the loss of turgor of the cells behind the cuticle, or (2) to the fact that the leaf-cells are killed, the phenomenon in the latter case not being capable of further analysis. With a view to deciding between these alternatives, attempts were made to see if penetration could be effected on leaves which were still alive.

The method of experiment was to take leaves of *Eucharis*, subject them to plasmolysis, inoculate some on the surface with *Botrytis cinerea*, the others being kept in a similar condition but uninoculated; then, when penetration was exhibited on the inoculated leaves, to recover the controls by washing out the plasmolysing liquid, and finally to show that the latter were again no longer penetrable. If this experiment could be carried out, it would prove that the change of penetrability was not associated with the living or non-living condition of the leaf tissue, but was associated with conditions of turgor in the leaf.

In carrying through this kind of experiment, there is considerable restriction in the choice of plasmolysing agent, on account of the necessity of recovering the control leaves from plasmolysis after penetration of the inoculated leaves is established. When the leaves were injected with Richards's solution made up to plasmolysing strength with cane sugar, penetration of the inoculated leaves took place, but only a small percentage (15–30 per cent.) of the control leaves could be recovered by washing out the plasmolysing liquid. Turnip extract of the full strength (osm. pr. = 0.39 molar cane sugar) gave better results, the plasmolysed inoculated leaves being penetrated, and 75 per cent. of the control leaves recovering on subsequent washing out with water. Cane sugar solutions of appropriate strength gave complete recovery, but when the spores were sown in drops of the sugar solution on the plasmolysed leaves, very feeble growth took place, and no penetration resulted.

It was desirable, in order to avoid changes of concentration due to diffusion, to use as far as possible the same liquid as plasmolytic agent and as nutrient. The addition of peptone (0.5 c.c. of 10 per cent. peptone to 5 c.c. of sugar solution) gave good results as regards growth, but the

¹ Richards's solution: cane sugar, 5 per cent.; KNO_3 , 1 per cent.; KH_2PO_4 , 0.5 per cent.; MgSO_4 , 0.25 per cent. Glucose-asparagin-mineral salts medium: glucose, 0.2 per cent.; asparagin, 0.2 per cent.; K_2PO_4 , 0.125 per cent.; MgSO_4 , 0.075 per cent.

addition of peptone to the plasmolysing liquid appeared to be deleterious, some of the controls failing to recover. The peptone was therefore used only in the infection drops, and the pure sugar solution was used for plasmolysis. The writers do not consider that chemotropic factors come into play at all, and even if it were so, it is against anticipation that the poorly nutritive cane sugar present in the intercellular spaces would attract the fungal germ-tubes from the more nutritive cane sugar-peptone medium in which they already are.

The following is an account of a particular experiment: A series of leaf portions were injected with cane sugar of concentrations varying from 0.1 to 1.0 molar at intervals equal to 0.1 M. Half of each set were kept as controls, and the other half inoculated on the upper surface with spores of *Botrytis cinerea*. In the case of the latter leaves, the spores were sown in drops of the particular cane sugar solution used for injection, with the addition of a little peptone solution as described above. The leaves injected with solutions of 0.4 M. and above showed plasmolysis. All the leaves were incubated at 20° C. After three days the inoculated leaves were examined for penetration. The leaves injected with 0.4 and 0.5 molar sugar were all penetrated, but none of the others, either above or below these concentrations, showed penetration. The control leaves were then washed for twelve hours in running water, by which time they had all recovered, i.e. they were again turgid and obviously alive. These recovered leaves were now inoculated in the usual way. After a further three days they remained unpenetrated, even though they were now somewhat yellow and obviously affected by the long exposure to unnatural conditions. The fact that the recovered leaves were able to resist penetration showed that the long exposure of the leaf to plasmolysis had not reduced the intrinsic resisting power of the cuticle.

Thus it appears in the present case that penetration of the injected leaves does not take place unless the injected liquid is sufficiently strong to plasmolyse the leaf cells. Further, it will be noted that when still stronger solutions are used (0.6 molar and upwards), penetration again fails, at any rate over a three days' incubation period. Whether penetration would later have taken place at some of the higher concentrations was not determined. This retarding or inhibiting effect of high concentrations, as the case may be, is easily understood. The retarding effect on fungal growth of high concentration of nutrient is a well-known experience, and was illustrated in the particular experiment just described by the fact that comparatively feeble growth had taken place after three days in the strongest nutrients tried. It is possible, however, to suggest another explanation of the effect in question, viz. that in these somewhat concentrated media the spores and hyphae of the fungus barely sink in the infection drops (on account of the high specific gravity of the nutrient liquid). Whether on account of the lack of sufficient

contact stimulus or for some other reason, it is noticeable that under such conditions the formation of attachment organs is to a large extent suppressed. This fact may explain the non-penetration in such a case. Failure to penetrate was similarly observed when spores were placed in a concentrated nutrient on *Allium* epidermis. In this case, however, it was found that, while no penetration took place within the limits of the drop, those hyphae which grew beyond the margin of the drop penetrated readily. This result lends some support to the view stated above in regard to the effect of high specific gravity of the nutrient liquid.

The foregoing results obtained with *Eucharis* and other leaves are certainly very difficult to explain on the basis of a chemical theory of penetration. If the hyphae of *Botrytis cinerea* are able to soften or dissolve the cuticle of *Eucharis* when the leaf is plasmolysed or killed (by the large variety of methods tested), then it is quite unintelligible why they should not be equally able to effect the same chemical action when the leaf is alive and turgid. The only possible basis of explanation is the mechanical theory of penetration. The further discussion of this point will be taken up later.

DISCUSSION.

To deal first with the question of the stimulus to penetration, it appears that the theory of chemotropism has many unsatisfactory features. Miyoshi's work, on which the theory is mainly based, has been considerably discounted in matters of fact by the later works of Fulton and Graves. Some of Miyoshi's own results, furthermore, are inconsistent with the view that penetration only takes place in response to chemotropic stimulation—for example, the experiments with membranes of paraffined cellulose. The results described in the earlier part of this paper clearly show that penetration, both of artificial and natural membranes, can take place in circumstances where any possible chemotropic factor is excluded. These experiments, it must be allowed, do not disprove the existence of chemotropism as a factor in membrane penetration—it is always possible that the effects observed might have been accentuated if conditions had been such as to allow of chemotropic stimuli playing a part. In other words, over and above the contact tropism which appears to the present writers to meet the case as an explanation of the penetration phenomenon, there may be superadded a definite positive chemotropism. The existence of the latter is as yet unsupported by critical experimental evidence, at least as far as the conditions governing membrane penetration are concerned. On the other hand, there is definite evidence that membrane penetration can take place under conditions in which, by exclusion of chemotropic influences, the only possible tropic factor is that due to contact. Thus it would seem to be the logical procedure to accept the theory of contact

tropism until such time as it is shown to be an insufficient explanation of the facts.

The *a priori* objection to the theory of contact tropism would seem to be that it affords little explanation of the specific relations existing between fungi and host plants. The chemotropic theory had considerable possibilities in this direction, as developed, for example, in a paper by Massee (6), but subsequent research has shown that the specific relation between the fungus and higher plant arises in general after the fungus has entered and not at the penetration stage at all.

It need hardly be pointed out that the theory of contact tropism does not necessitate the conclusion that any fungus placed on any host plant would be able to penetrate, even assuming the particular fungus to be sensitive to contact stimulation. The capacity of the fungus to germinate in the nutrient available on the host surface, and that to overcome the resistance of the cuticle, are factors of importance, and it is by the interplay of these factors that one could explain such specific relationships as are concerned in the process of penetration.

It may be added that while, according to the views put forward in this paper, chemical substances issuing from the host plant into the infection drop have no directive effect on the fungal hyphae, they are not therefore to be considered as of no importance to the fungus. Such substances may affect the germination and growth of the fungus, i. e. act as a purely environmental factor.

When we come to consider the relative merits of the mechanical and chemical theories of the penetration process itself, we find a very similar state of affairs to that already dealt with in the case of the directive stimulus to penetration. That is, that while the only direct evidence brought forward favours the mechanical theory, the latter is not generally considered by mycologists to afford an adequate explanation. There is first of all the lack of specificity which the mechanical theory seems to entail—this has already been dealt with—and there is, further, the difficulty of an apparently soft structure like a fungal hypha penetrating a rigid protective layer like a plant epidermis. The latter objection would seem to be much discounted by the fact that fungal hyphae have been shown to penetrate paraffin wax, collodion membranes, and even gold-leaf. In all these cases penetration must have been effected by mechanical action alone, and if, as must be accepted from the experimental evidence, fungi are able to penetrate mechanically the substances mentioned, there is clearly no reason on *a priori* grounds why they should not be capable of similarly penetrating a plant epidermis.

In the present series of studies the mechanical theory was adopted in the first instance from the fact that there was no evidence of the existence of any substance in the germ-tubes of *Botrytis cinerea* capable of attacking

cuticle (2). An extract was obtained which rapidly attacked the tissue when injected into it, but which had no action when simply placed on the surface. Here again one cannot deny the existence of a cuticle-dissolving enzyme, even for the case of *Botrytis cinerea*, as the technique of extraction may have been insufficient, or the enzyme may even be non-extractable. The natural procedure, however, must be to accept the factor which is known to exist, and see how far the various data can be explained on its basis.

It may be mentioned that an objection of a general nature has been raised (in discussion) against the view put forward here that penetration takes place by mechanical means and not through the agency of a cutin-

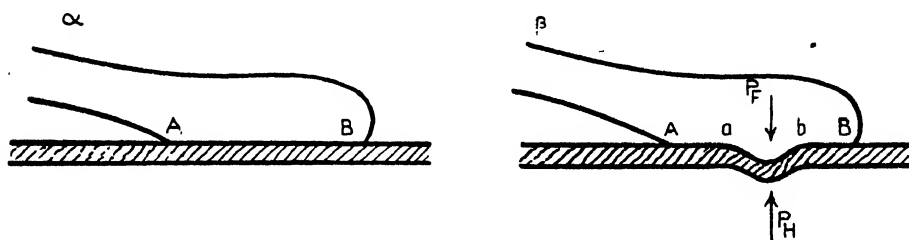


Diagram illustrating the mechanics of epidermal penetration by a fungus. For description, see text.

dissolving enzyme. The objection is that cutinized material does disappear in nature, a fact which perhaps indicates that cutin-dissolving enzymes do exist, even though no one has succeeded in extracting them. It is well known, however, that cutinized material is of a very resistant nature, and that it disappears very slowly even under conditions highly favourable to decomposition, such as obtain, for example, in moist soils, where it is subjected to the combined action of a great variety of micro-organisms. This slow decomposition of cuticle is illustrated very strikingly in the observation of Priestley (11) that certain peat deposits consist to a large extent of cuticular, endodermal, and corky tissues of heath-inhabiting plants. Such being the case, it is not at all obvious that the slow decomposition of cuticle in nature, even if it is brought about by the action of fungal enzymes, in any way suggests the means by which a parasitic fungus such as *Botrytis cinerea* is able to penetrate the cuticle of a suitable plant within a few hours of its being placed in contact with the latter.

The experiments on *Eucharis* leaves described on pp. 652-6 give strong support to the mechanical theory of penetration. They show further that the total resistance to penetration of the cuticle is partly a function of the cuticle itself and partly due to turgor of the cells supporting the epidermis. The mechanics of the process of penetration, as the present writers see it, will be made clearer by reference to the figure above, which repre-

sents diagrammatic sections through an attachment organ from which a penetration hypha is being sent out. Attachment takes place over the area indicated in section by the line AB. The smaller area ab is that from which the growth takes place which leads to rupture of the epidermal wall. The mechanical reaction incidental to pressure over the area ab is taken up by adhesion over the annular area represented by $Aa-Bb$. The effect of growth over the area ab will be a depression of the epidermal wall such as is indicated in β , the epidermal wall being now under greater tension. The equation of equilibrium of the distorted portion ab of the epidermal wall is of the following form :

$$P_F = T + P_H,$$

where P_F is the pressure due to the fungus; P_H is the hydrostatic pressure of the underlying host cell; and T is a complex function dependent upon the tension of the epidermal wall, the degree of distortion of the portion ab , the area of the latter, &c. The condition for successful penetration is that

$$P_F > T' + P_H,$$

where T' is the limiting value of the function T above defined, i. e. it is the value attained by the latter when the epidermal wall is strained to breaking point. The results obtained with *Eucharis* leaf may be therefore explained on the assumption that for that particular case

$$\begin{aligned} P_F &< T' + P_H \\ \text{but } P_F &> T'. \end{aligned}$$

The quantity P_H , which has been defined as the hydrostatic pressure in the underlying epidermal cell, will in general be identical with the osmotic pressure of the same cell. If the cell should happen to be somewhat flaccid at the time when the infection drop is placed on the overlying surface, it will rapidly absorb water through the epidermis until the limiting osmotic pressure is reached.

It remains to discuss the nature of the force P_F . The assumption that this quantity is the osmotic pressure of the fungal cell leads to the conclusion, as can be shown by a simple calculation, that the tensile strength of the fungal cell wall is much greater than that of the plant epidermis. Though nothing definite is known as regards the tensile strength of either epidermis or fungal cell wall, it is nevertheless very difficult to believe that such is the case. A much more reasonable hypothesis is as follows :

The turgor of the fungal cell does not produce the pressure required for penetration, but merely supplies the conditions necessary for growth of the infection hypha. The latter, represented in the figure by the cone with base ab , grows by absorption of cell-wall material along the sides of the cone. This obviously leads to lengthening of the cone, with resultant stretching

and final rupture of the portion of epidermis in contact with it. The extreme minuteness of this cone ensures its great mechanical rigidity, so that the back thrust of the deformed epidermis is taken up, not by the osmotic pressure of the fungus, but by the rigid cell wall of the latter. It is obvious that the pressure exerted by the fungal penetration hypha will be greatest at the apex of the cone, where in consequence rupture will take place. The infection hypha passes through as a fine thread, the pectinase enzyme of the fungus is now able to act, and all difficulty of penetration as far as the mechanics of the process is concerned disappears.

In the foregoing, one explanation has been given of the observed fact that a turgid leaf is more difficult to penetrate than a flaccid one. The following is a further consideration. The conical depression represented by *ab* is not mechanically strong until it deepens, and therefore the most difficult stage in the penetration process is the first one, viz. when the epidermal surface is just being deformed. It is obvious that this difficulty will be more readily overcome when the epidermis is initially slack, as in the flaccid leaf, than when it is taut, as in the turgid leaf.

It may similarly be suggested that the well-known tendency of fungi to penetrate along the line of junction of epidermal cells rather than over the epidermal cell itself is due to the initial concavity of the epidermal surface in the former case.

The observation that *Eucharis* epidermis is readily penetrated by *Botrytis* germ-tubes only when it is backed by flaccid tissue raises the question as to whether plants may be more readily attacked when in the wilted condition than when turgid. Such a result was claimed by Nordhausen (10) and more recently by Rivera (13). The latter confined barley plants under a bell-jar and produced partial wilting by exposing the plants suddenly to direct sunlight. Under these conditions a greater susceptibility to infection by oidiospores of *Erysiphe graminis* was observed. In experiments with *Eucharis* leaves along similar lines, the present writers failed to establish a like effect. Spores of *Botrytis cinerea* were sown in dilute nutrient on leaves which had been allowed to wilt in the air until they were thoroughly flaccid. Penetration, however, did not take place, and it was found that the portion of leaf underlying the infection drop recovered its turgor by absorption of liquid from the infection drop. This recovery took place within five to ten hours from the time of inoculation, that is, too soon to allow penetration. Even when the wilted leaf was placed in a dry atmosphere, and the infection drop protected from evaporation by a ring and coverslip, the drop shrank in a few hours and the tissue immediately below became again turgid. Rivera's results are probably to be explained on the ground that penetration of the barley leaf by the spores of the mildew requires a much shorter time than is necessary in the case of *Botrytis* spores on the *Eucharis* leaf, and partly also to the fact that the infection

drops in the former case only slightly wet the surface of the host plant. In these circumstances the flaccid condition of the host cells underlying the infection drop may persist long enough to allow of infection being established.

The experiments with epidermal 'windows' of *Eucharis* leaf recall a similar series of experiments described by Salmon (14). The latter found that biologic strains of *Erysiphe graminis* were able to attack otherwise immune species of grasses when the tissue of the latter was damaged in a variety of ways, e. g. by shaving off some of the underlying tissue while the inoculated surface of the host was left intact in the form of an epidermal 'window'. The analogy between Salmon's experiments and those of the present paper is, however, not clear. In the case of specialized parasites, such as the mildews, it is generally understood that the resistance of a particular species of host plant to the 'wrong' biological form of the parasite lies, not in resistance to cuticular penetration, but to failure of the haustoria to develop normally after the fungus has entered. Salmon's results indicate that the leaf injury produces some physiological change in the neighbouring living cells of the host, with the result that the latter are enabled to establish a symbiotic condition with the parasite. In the present experiments with a non-specialized parasite like *Botrytis cinerea*, no such considerations arise, and the results are to be explained on the basis of cuticular penetration only.

SUMMARY.

1. Membranes of paraffin wax, which were shown to be impermeable to one of the simplest electrolytes, were readily penetrated by germ-tubes of *Botrytis cinerea*, provided sufficient nutrient was available to the spores to allow of good germination. Penetration in this case could not have been determined by any chemotropic influence acting across the membrane.

2. Membranes of formalized gelatin, within limits of hardness, were likewise penetrated. These membranes are highly permeable to crystalloidal substances. It was found that penetration by the fungal hyphae took place with equal freedom independently of the original distribution of the nutrient material.

3. Membranes prepared from the epidermis of *Allium* scales and of *Eucharis* leaf were readily penetrated. The removal by thorough washing of any possible chemotropic substances present in the epidermal cells had no effect on the penetrability of these membranes by the fungus. Penetration took place with the same facility from the inner surface of these epidermal membranes as from the outside.

4. Experiments with a series of membranes of formalized gelatin, the hardness of which can be varied, showed that different fungi possess different intrinsic powers of penetration. The use of such a series of membranes

would enable a rough standardization of the penetrative power of different fungi.

5. Germ-tubes of *Botrytis cinerea* are unable to penetrate the epidermes of *Eucharis* spp. and of certain other plants so long as the underlying leaf tissue is turgid. When the turgor of the underlying cells is removed by plasmolysis or by any form of killing, the epidermis is readily penetrated.

6. A discussion is given in which it is pointed out that the only satisfactory theory of membrane penetration by fungi is that (a) the stimulus to penetration is one of contact, (b) the means of penetration is purely mechanical.

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The Mating Method of Identification of a *Coprinus* growing on Germinating Seeds of Mangel and Sugar-beet.

BY

A. H. REGINALD BULLER

AND

DOROTHY E. NEWTON.

With Plate XXVIII and six Tables in the Text.

I. INTRODUCTION.

IN 1914 Mr. Raymond Finlayson, of the Seed Testing Laboratory, Wood Green, London, observed a *Coprinus* coming up on germinating seeds¹ of Mangel, Beet, and Sainfoin, and he sent some of the material to Mr. Carleton Rea. Mr. Rea regarded the fungus as *Coprinus pilosus*, Beck,² but sent some of the seeds to the senior author (A. H. R. B.) with a request for his opinion. The *Coprinus* duly came up on some of the moistened seeds and from its morphological characters it was identified by Buller as *Coprinus lagopus*, Fr., and it was considered by him as identical with the form of *C. lagopus* which occurs very commonly on horse-dung both in England and Canada.³

In 1919 Pape⁴ published a note on the occurrence of a *Coprinus* on germinating Mangel and Beet seeds. His attention had been called to the matter by Dr. Zade, of Jena. Pape observed that the fungus grew almost invariably on the seed-balls (pericarps) themselves, and he observed nothing to suggest that the fungus is a parasite. He says: 'The question of the significance of the fungus in respect to the Beet seed upon which it grows

¹ Mangel and Beet seeds, when sown, are enclosed by a rough pericarp. The *Coprinus* under discussion grows on the pericarp.

² Carleton Rea, *in litt.*

³ A. H. R. Buller: Researches on Fungi, London, vol. iii, p. 308, 1924. The *Coprinus* obtained from the Mangel seeds is illustrated in Fig. 141, D, p. 320.

⁴ Pape: *Coprinus* auf Rübensamen. Mitteilungen a.d. Biol. Reichsanst. f. Land- und Forstwirtschaft, Heft 17, pp. 13-16, 1919, with two illustrations.

must be left open. A lowering of the germinating power of the seed due to the fungus or damage to the young seedling from it has not been observed. The fact that the fungus is most abundant upon seed which we found to have a low germinating power may be due to the fungus finding more nourishment in seeds which have failed to germinate than in those which produce seedlings.' Pape identified the fungus as *Coprinus nycthemerus*, Fr.

In 1926 the *Coprinus* was observed by Mr. W. A. Dillon Weston, of the School of Agriculture at Cambridge, England, coming up on Mangel seeds, and by Mr. M. W. Gardner, of the Agricultural Experiment Station at Purdue University, Lafayette, U.S.A., coming up on Sugar-beet seeds; and each of these investigators sent a packet of his infected seeds to the senior author of this paper (A. H. R. B.) with a request for an identification of the fungus. Thereupon the work about to be described was undertaken.

New *Coprinus* fruit-bodies were obtained by the writers from (1) the Mangel seeds, and (2) the Sugar-beet seeds, by sowing the seeds on sheets of wet filter-paper in a crystallizing dish. Some of the seeds germinated normally, whilst others did not germinate at all. On the latter, in both cultures, after a lapse of about three weeks, several *Coprinus* fruit-bodies appeared (Pl. XXVIII). The *Coprinus* on the Mangel seeds was identical in appearance with the *Coprinus* on the Sugar-beet seeds, and both Coprini had all the morphological and growth characteristics of *Coprinus lagopus*, Fr., as described and illustrated in vol. iii of Buller's 'Researches on Fungi'.¹

Coprinus lagopus is of very common occurrence on horse-dung balls both in England and in Canada, and it can almost always be procured by putting fresh horse-dung balls in a covered crystallizing dish and leaving them there for two or three weeks at room temperatures.

The recent advance in our knowledge of the sexual process in the Coprini permits of an experimental verification of such an identification as has just been made. The experimental method of identification may be called the *mating method*; and, in what follows, it will be treated of both in theory and in practice.

II. THE MATING METHOD.

When monosporous mycelia derived from spores of a *single* fruit-body of *Coprinus lagopus* are paired in all possible ways on dung-agar plates and the subsequent development or non-development of clamp-connexions is taken as a criterion of a positive or of a negative sexual reaction respectively, it is found that the mycelia fall into *four* sexual groups, which may be expressed in Mendelian symbols as follows: (AB), (ab), (Ab), and (aB).

¹ A. H. R. Buller: loc. cit., pp. 299-327, Figs. 130-47.

Thus Hanna¹ with ten monosporous mycelia from as many spores of a single wild fruit-body, which he paired in all possible ways, obtained the results embodied in Table I. In this table a (+) sign indicates that clamp-connexions appeared in the compound mycelium and a (-) sign that they did not. The numbers 50-59 were arbitrary numbers given to particular mycelia. After the results of pairing had been obtained, the table was rearranged so as to bring like mycelia together, and the Mendelian symbols required to explain the reactions were then added.

	AB				ab				Ab		aB
	51	52	54	55	57	58	59	50	56	53	
AB	51	—	—	—	+	+	+	+	—	—	
	52	—	—	—	+	+	+	+	—	—	
	54	—	—	—	+	+	+	+	—	—	
	55	+	+	+	—	—	—	—	—	—	
ab	57	+	+	+	—	—	—	—	—	—	
	58	+	+	+	—	—	—	—	—	—	
	59	+	+	+	—	—	—	—	—	—	
Ab	50	—	—	—	—	—	—	—	—	+	
	56	—	—	—	—	—	—	—	—	+	
aB	53	—	—	—	—	—	—	+	+	—	

TABLE I. *Coprinus lagopus*. All possible pairings of ten monosporous mycelia obtained from the spores of a single Vancouver fruit-body. A similar table is obtained when ten monosporous mycelia derived from ten spores of one fruit-body are paired in all possible ways with ten monosporous mycelia derived from ten spores of another fruit-body, provided the two fruit-bodies belong to one and the same geographical strain.

In Table I it will be observed that the ten monosporous mycelia fall into *four* groups.

When monosporous mycelia derived from spores of a fruit-body of *one* so-called geographical strain of *Coprinus lagopus* are paired in all possible ways with monosporous mycelia derived from spores of another fruit-body of the *same* geographical strain, it is found that, as before, the mycelia fall into four sexual groups to which may be given the Mendelian symbols (AB), (ab), (Ab), and (aB). This was proved by Hanna,² whose table of results for matings of the kind under discussion resembles Table I.

When monosporous mycelia derived from a fruit-body of *one* so-called geographical strain of *Coprinus lagopus* are paired in all possible ways with the monosporous mycelia of *another* geographical strain, perfect fertility

¹ W. F. Hanna: The Problem of Sex in *Coprinus lagopus*. Ann. Bot., vol. xxxix, p. 436, 1925.

² Ibid., p. 443.

results, i. e. clamp-connexions appear in every plate. Thus Hanna¹ mated eleven monosporous mycelia, derived from as many spores taken from an Edmonton (Province of Alberta) fruit-body, in all possible ways with eleven monosporous mycelia derived from as many spores taken from a Winnipeg (Province of Manitoba) fruit-body, with the results shown in Table II.²

		A^4B^4		a^4b^4		A^4b^4		a^4B^4				
		4	7	8	5	2	6	10	11	1	3	9
A^2B^2	25	+	+	+	+	+	+	+	+	+	+	+
	26	+	+	+	+	+	+	+	+	+	+	+
	27	+	+	+	+	+	+	+	+	+	+	+
	28	+	+	+	+	+	+	+	+	+	+	+
a^2b^2	20	+	+	+	+	+	+	+	+	+	+	+
	23	+	+	+	+	+	+	+	+	+	+	+
	24	+	+	+	+	+	+	+	+	+	+	+
	21	+	+	+	+	+	+	+	+	+	+	+
a^2B^2	29	+	+	+	+	+	+	+	+	+	+	+
	30	+	+	+	+	+	+	+	+	+	+	+
A^2b^2	16	+	+	+	+	+	+	+	+	+	+	+

TABLE II. *Coprinus lagopus*. The pairing of eleven monosporous mycelia of an Edmonton fruit-body (No. 2) with eleven monosporous mycelia from a Winnipeg fruit-body (No. 4).

Table II contains nothing but (+) signs, which indicates that clamp-connexions appeared in every pairing.

When monosporous mycelia derived from the spores of a fruit-body of *one species* of *Coprinus* are paired with monosporous mycelia derived from the spores of *another* morphologically distinct species of *Coprinus*, there is complete infertility. Thus the authors made pairings between *Coprinus lagopus* and *C. macrorhizus*, between *C. lagopus* and *C. Rostrupianus*, and between *C. Rostrupianus* and *C. macrorhizus*, with the results shown in Tables III, IV, and V respectively.

Tables III, IV, and V contain no (+) signs, thus showing that no clamp-connexions were developed in any of the pairings. The absence of

¹ Loc. cit., p. 441.

² Similar results have been recorded for *Schizophyllum commune* (Kniep), *Coprinus Rostrupianus* (Newton), *C. comatus* (Brunswick), *C. radians* (Vandendries), and *Panaeolus campanulatus* (Vandendries); but, recently, Vandendries has found an exception to this rule in some strains of *Coprinus micaceus* which were sterile with one another (R. Vandendries, L'hétérohomothallisme et la stérilité entre races géographiques de *Coprinus micaceus*, in Mém. de l'Acad. roy. de Belgique, tom. ix, pp. 42-50, 1927).

clamp-connexions clearly indicates a failure in the attempt to produce *Coprinus* hybrids.

The difference in the results obtained by mating (1) monosporous mycelia of *Coprinus lagopus* derived from a single fruit-body or from two fruit-bodies of one and the same geographical strain, (2) monosporous mycelia of *Coprinus lagopus* derived from two geographical strains, and

		<i>C. macrorhizus.</i>			
		1	2	3	4
<i>C. lagopus.</i>	I	—	—	—	—
	II	•	—	—	—
	III	—	—	—	—
	IV	—	—	—	—

TABLE III. The pairing of four monosporous mycelia of *Coprinus lagopus*, Fr., with four monosporous mycelia of *C. macrorhizus*, (Pers.) Rea.

		<i>C. Rostrup.</i>		
		1	2	3
<i>C. lagopus.</i>	I	—	—	—
	II	—	—	—
	III	—	—	—
	IV	—	—	—

TABLE IV. The pairing of four monosporous mycelia of *Coprinus lagopus*, Fr., with three monosporous mycelia of *C. Rostrupianus*, Hansen.

		<i>C. macrorhizus</i>			
		1	2	3	4
<i>C. Rostrupianus</i>	I	—	—	—	—
	II	—	—	—	—
	III	—	—	—	—
	IV	—	—	—	—

TABLE V. The pairing of four monosporous mycelia of *Coprinus Rostrupianus*, Hansen, with four monosporous mycelia of *C. macrorhizus*, (Pers.) Rea.

(3) monosporous mycelia derived from two morphologically distinct species of *Coprinus* has now been set forth. Using this difference as a guide, an attempt was made to determine whether or not the *Coprinus* which occurs on Mangel seeds at Cambridge, England, and on Sugar-beet seeds at Lafayette, U.S.A., are geographical strains of one and the same species, and, in particular, of *Coprinus lagopus* as it occurs on horse-dung at Winnipeg, Canada.

III. EXPERIMENTAL RESULTS.

Three monosporous mycelia were obtained from three spores derived from each of the following: (1) a *Coprinus* fruit-body which grew on Cambridge Mangel seeds; (2) a *Coprinus* fruit-body which grew on Lafayette Sugar-beet seeds; and (3) a *Coprinus lagopus* fruit-body which

came up spontaneously on horse-dung gathered at Winnipeg. These nine monosporous mycelia were then paired on dung-agar plates in all possible ways, with the results embodied in Table VI.

<i>C. lagopus</i>			<i>Mangel</i>			<i>Sugar-beet</i>		
1	2	3	1	2	3	1	2	3
<i>C. lagopus</i>	1	—	—	+	+	+	+	+
	2	—	—	—	+	+	+	+
	3	+	—	—	+	+	+	+
<i>Mangel</i>	1	+	+	+	—	—	—	+
	2	+	+	+	—	—	—	+
	3	+	+	+	—	—	—	+
<i>Sugar-beet</i>	1	+	+	+	+	+	+	—
	2	+	+	+	+	+	+	—
	3	+	+	+	+	+	+	—

TABLE VI. The pairing of nine monosporous mycelia, three derived from a Winnipeg *Coprinus lagopus* fruit-body, three derived from a Cambridge Mangel-seed *Coprinus* fruit-body, and three derived from a Lafayette Sugar-beet-seed *Coprinus* fruit-body.

In Table VI, in the pairings *Coprinus lagopus* × *Coprinus lagopus*, Mangel × Mangel, and Sugar-beet × Sugar-beet, the results, as was to be expected, are essentially like those shown in Table I; but the pairings were too few to give all the four possible groups of mycelia (AB), (ab), (Ab), (aB). However, in the pairings of *C. lagopus* × *C. lagopus* and Sugar-beet × Sugar-beet, it is obvious that three of the four possible groups of mycelia are represented (cf. Table I). In the pairings Mangel × Mangel, since no clamp-connexions appeared in any pairing, the three mycelia may have all belonged to one of the four possible groups, or one of the mycelia may have belonged to one group, e.g. (AB), and the two other mycelia to another group, e.g. (Ab) or (aB).

In Table VI complete fertility is shown in all of the following pairings: *Coprinus lagopus* × Mangel, *Coprinus lagopus* × Sugar-beet, and Mangel × Sugar-beet. These results, which resemble those of Table II, indicate that the *Coprinus* occurring at Cambridge, England, on Mangel seeds, the *Coprinus* occurring at Lafayette, U.S.A., on Sugar-beet seeds, and *Coprinus lagopus* occurring on horse-dung at Winnipeg, Canada, are merely three so-called geographical strains of one and the same species, namely, *Coprinus lagopus*, as described in vol. iii of Buller's 'Researches on Fungi'.

IV. DISCUSSION.

As we have seen, the *Coprinus* on Mangel seeds was regarded by Mr. Carleton Rea as *C. pilosus*, Beck, and by Pape as *C. nycthemerus*, Fr., whereas we identify it as *Coprinus lagopus*, Fr., as very fully described and illustrated with numerous photographs and drawings in Buller's 'Researches on Fungi'.¹

It seems highly probable that *Coprinus pilosus* and *C. nycthemerus*, as understood by Rea and Pape respectively, are but synonyms for *C. lagopus*, as understood by us. Pape² gives not only some illustrations but also a description of his fungus which enables us to perceive that his fungus and ours are in all probability one and the same species, and he adds: 'The foregoing characters of the fungus point to *Coprinus nycthemerus*, Fr., as being nearest to it. At the same time, a comparison between the present fungus and the figures given by Brefeld and Cooke of *C. nycthemerus* leave it uncertain whether the two are actually identical.' The diversity of opinion as to what the Mangel-seed fungus should be called is doubtless due to the fact that the original descriptions of many of the smaller Coprini are so brief and incomplete as to be little more than useless for identification purposes. Exactly what species of *Coprinus* are to be identified with *C. pilosus* and *C. nycthemerus* is not clear to the writers. *Coprinus pilosus* may well be a synonym for *C. lagopus*. The illustrations of *C. nycthemerus* as given by Brefeld,³ Cooke,⁴ and Ricken⁵ do not resemble one another and do not suggest the *Coprinus* shown in our photographic illustration. Fries⁶ and Rea⁷ state that *C. nycthemerus* is uncommon, and Lange⁸ in his monograph of the Danish Coprini does not even mention it. On the other hand, *Coprinus lagopus*, as understood by us, is an exceedingly common fungus in both Europe and North America. One of us (A. H. R. B.) has observed it many times on the under side of horse-dung plats in fields in England, and the ease with which the fungus may be obtained in fresh horse-dung cultures in both England and Canada goes to show that its spores must be widely dispersed in the pastures of both these countries.

As we have seen, the mating method has enabled the writers to determine with certainty that three Coprini obtained from as many different countries belong to one and the same species. There seems no reason why

¹ A. H. R. Buller: loc. cit.

² Pape: loc. cit., pp. 14-15.

³ O. Brefeld: Untersuchungen über Pilze, Leipzig, Heft viii, 1909, Pl. XII, Figs. 10, 11.

⁴ M. C. Cooke: Illustrations of British Fungi, London, Pl. 682, B.

⁵ A. Ricken: Die Blätterpilze, Leipzig, 1915, Taf. xxi, Fig. 5.

⁶ E. Fries: Hymenomycetes Sueciae, Upsala, 1857, p. 466.

⁷ C. Rea: British Basidiomycetae, Cambridge, 1922, p. 511.

⁸ J. E. Lange: Studies in the Agarics of Denmark. Dansk Botanisk Forening, 1915, Part II, *Coprinus*, pp. 32-50.

the same method should not be used to establish the identity of other heterothallic Hymenomycetes, physiological experiment thus directly helping the systematist.

V. SUMMARY.

1. As shown by comparative morphology and by mating experiments, the *Coprinus* which comes up on germinating Mangel seeds in England and on germinating Sugar-beet seeds in the United States of America is identical with *Coprinus lagopus*, Fr., as it occurs on horse-dung in Canada.

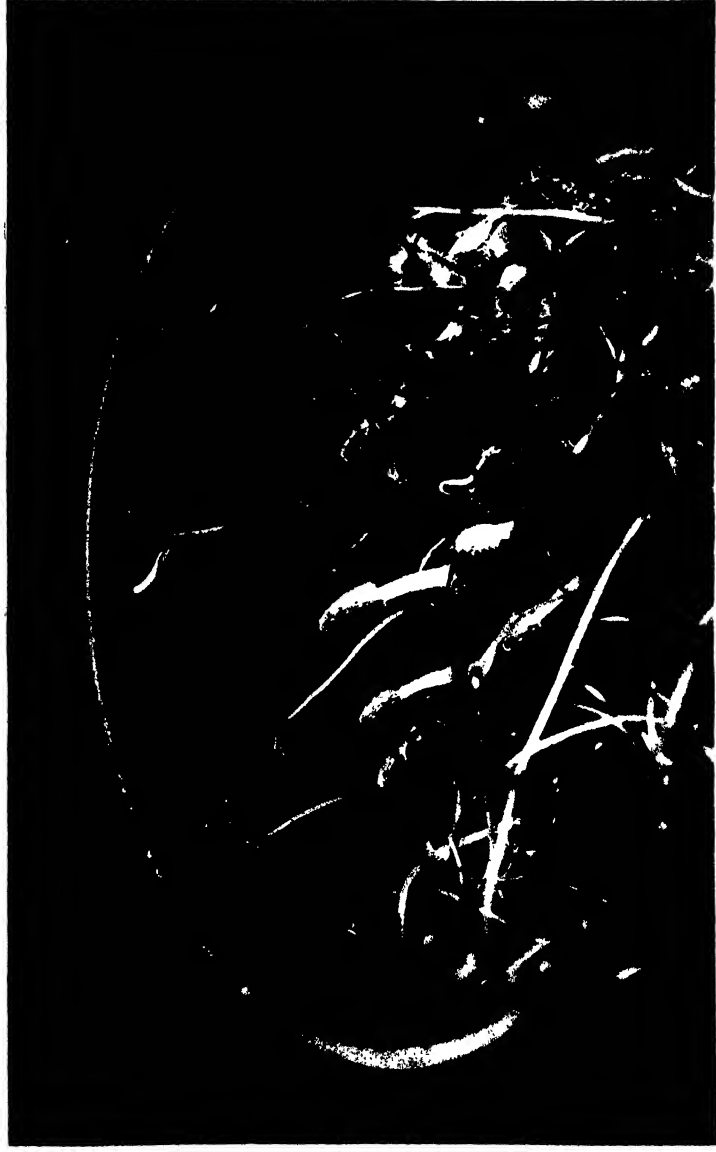
2. *Coprinus lagopus* is not confined to a substratum of seeds, for it is a fungus of wide occurrence on horse-dung in pastures both in Europe and North America.

3. The mating method may be used to assist the systematist in deciding specific identity in the heterothallic Hymenomycetes in general.

EXPLANATION OF PLATE XXVII.

Illustrating the paper of Professor A. H. R. Buller and Miss D. E. Newton on the Mating Method of Identification of a *Coprinus*.

Photograph of fruit-bodies of *Coprinus lagopus* growing on Sugar-beet seeds (fruits) obtained from Lafayette, Indiana, U.S.A., about three weeks after the seeds had been placed on wet filter-paper in a crystallizing dish. Some of the seedlings have developed normally. Natural size.



BULLER & NEWTON—IDENTIFICATION OF COPRINUS

Huth coll.

Physiological Anatomy of the Irritable Organs of some Climbing Plants.

BY

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AND

R. H. DASTUR,

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With Plate XXIX.

OF all the different types of induced movements which are of widespread occurrence in plants, the thigmotropic movements occurring in climbing plants have been the subject of investigation for a long time past. The physiology of these movements has been studied by various botanists, including Fitting, Pfeffer (8), Sachs, and Darwin (1), and the mechanism of curvature has been explained by them in many cases. The curvatures are either caused by an unequal growth on the two sides of an organ or are due to the contraction of the side in contact with the support. In some cases they are due to both causes.

The thigmotropic movements are caused by some specialized tissues which show structural peculiarities in accordance with their function. Certain cells act as organs of perception of a stimulus, while others respond to it. The histology of the tendrils of some climbing plants has been studied by MacDougal (4), Penhallow (7), Worgitzky (9), Müller (6), and Lisk (3), and the anatomical peculiarities of the tissues responsible for the curvatures have been pointed out by them. Haberlandt (2) has described many types of specialized tissues in different plants. He has described the epidermal cells which act as organs of perception, and motor tissues which respond to the stimulus of contact.

The present investigation has been undertaken with a view to study the histology of climbing organs and to locate and describe the structural

features of tissues which cause the grasping or twining movements of plants.

The following plants belonging to four different families have been studied: *Thunbergia fragrans*, Roxb.; *T. alata*, Boj.; *T. laurifolia*, Lindl.; *T. mysorensis*, T. Anders.; *Vitis trifolia*, L., *V. latifolia*, Roxb.; *V. quadrangularis*, Wall.; *V. discolor*, Dalz.; *V. elongata*, Wall.; *Luffa aegyptiaca*, Mill.; *Luffa acutangula*, Roxb.; *Passiflora Munroii*, Mast.; *P. kermesina*, Link & Otto; *P. minima*, L.; *P. lunata*, Wild.

Efforts have been made to find a suitable stain which would properly stain the cells, and out of the twenty-seven stains and their combinations that were tried gentian violet was found to be the best, as it brings out the minute details very clearly. It is used either as a single stain or in combination with bismarck brown.

On examining the structure of the irritable organs of the above-mentioned plants it is noticed that there are many histological features common to all. These structural peculiarities fall under three tissue systems and are discussed under separate headings below.

Sensory tissue system. There is considerable evidence that the stems and roots possess the power of perceiving the direction of gravity on account of the presence in their cells of specifically heavier bodies known as statoliths. These bodies cause the positive and negative geotropic curvatures in these organs when they are displaced from their normal position of geotropic equilibrium. In the case of twining stems, the twining movements come about by the revolving movements of their apices and negative geotropism necessary for the straightening of the twined region. The revolving movements of the stems are, according to some authors, purely autonomous, while it is held by others that gravity has a determining influence upon it.

In the twining stems of the species of *Thunbergia* the endodermal cells with distinct casparian bands appear to function as statocysts, as they contain very conspicuous starch grains which are always found on the physically lower sides of the cells, whether they are vertically inclined or horizontal. The presence of statoliths and their orientation inside the cells indicate that gravity may have a determining influence on the revolving movement (Pl. XXIX, Fig. 8).

In plants which climb by means of their tendrils, the epidermis of the latter functions as the sensory tissue, and it shows structural modifications in accordance with this special function. In the plants investigated different stages of the progressive modification are seen. As found in the species of *Passiflora* (Pl. XXIX, Fig. 5) a rough external epidermal surface is the first step towards the development of the structural peculiarity for the perceptive faculty. In the species of *Vitis* the unevenness is more pronounced, and takes the shape of corrugations (Pl. XXIX, Figs. 3, 4, 6). The corrugations

appear as ridges on the external surface in species of *Thunbergia*, and the layer of protoplasm is seen to penetrate into the ridges in the outer walls of the epidermal cells (Pl. XXIX, Figs. 1, 2, 7).

The epidermal cells of *Thunbergia fragrans*, Roxb., and *Luffa aegyptiaca*, Mill., show the most advanced stage of specialization. There are distinct pits in the outer walls of the epidermis, and the protoplasmic layer is seen to penetrate the cavities of the pits. The pits are found only on the sensitive abaxial side of the tendrils of *Luffa aegyptiaca* (Pl. XXIX, Fig. 10, *a, b*).

Motor tissue system. Various kinds of autonomous movements are performed by plant organs with the help of living motor tissues which are in some cases restricted to definite plant organs. Haberlandt (2) has shown in the tendrils of *Urvillea ferruginea* that the curvature of the tendrils is caused by the contraction of motor cells on the concave side. MacDougal (5) has also described a similar phenomenon in *Passiflora coerulea*.

The cortical cells in the tendrils of the species of *Passiflora* resemble in structure and in behaviour the motor cells described by Haberlandt (2). Similarly, the cortical cells in the tendrils of *Luffa aegyptiaca*, Mill., *L. acutangula*, Roxb., *Vitis trifolia*, L., and *V. latifolia*, Roxb., behave as motor cells.

Mechanical tissue system. The climbing plants in general require requisite mechanical strength as they are subjected to various kinds of tensions. The stems of twining plants have to guard against longitudinal tensions arising from growth in length and in thickness of a supporting stem, and consequently the mechanical system of twining stems has a centripetal tendency. In some cases the mechanical system is centralized, as is the case in other inextensible organs such as roots and rhizomes.

The spirally coiled tendrils of some climbing plants need a certain amount of rigidity in a single plane only, as they have a bilateral or dorso-ventral symmetry. A mechanical girder with two flanges is necessary, one on the convex side to act as a 'compression flange', and the other on the concave side as a 'tension flange' if the supporting stem grows in length or in thickness. The disposition of the mechanical elements in cucurbitaceous plants is described by Worgitzky (9).

The tendrils of some plants are at first radial in symmetry, but become bilateral owing to the greater development of wood on the concave side than on the convex side. They show the same arrangement of the mechanical tissue. The tendrils of other plants remain radial in symmetry, and they resemble the twining stems in the disposition of the mechanical tissue.

A tabulated list showing the disposition of the mechanical tissue in the climbing organs of plants studied is given below.

Twining Stems.

<i>Name.</i>	<i>Mechanical Tissues.</i>	<i>Remarks.</i>
<i>Thunbergia.</i>	Subepidermal zone of sclerenchyma and wood fibres.	Centripetal tendency. The strength of the tissue increasing towards the centre.

Tendrils with Bilateral Symmetry.

<i>Luffa.</i>	Sclerenchymatous band on the concave side.	Acts as a tension flange.
	Two smaller bands of the same on the convex side.	Act as a compression flange.
	Lignification of the cortex on the convex side.	
	Lignification of the pith.	Makes tendril inflexible.

Tendrils with Radial Symmetry but becoming Bilateral.

<i>Vitis trifolia</i> , L.	Secondary wood fibres and turgescerent parenchyma, with bast fibres in addition in <i>V. latifolia</i> , Roxb., on the concave side.	They act as a tension flange.
<i>V. latifolia</i> , Roxb.		
	Bast fibres on the convex side.	Compression flange.
<i>Passiflora Munroii</i> , Mast.	Secondary wood fibres on the concave side.	The same arrangement as in twining stems.
<i>P. kermesina</i> , Link and Otto.	Bast fibres on all sides except <i>P. minima</i> , L., on the concave side only.	The change to bilateral symmetry is accompanied by additional mechanical elements in the form of wood fibres on the concave side.
<i>P. minima</i> , L.	Pith lignified.	
<i>P. lunata</i> , Wild.		

Tendrils with Radial Symmetry.

<i>V. quadrangularis</i> , Wall.	Collenchymatous ring or groups in the cortex. Bast fibres or rings of sclerenchyma round the vascular ring.	Centripetal tendency of the mechanical tissue.
<i>V. discolor</i> , Dalz.		

SUMMARY.

1. In the different plants studied, stages in the modification of the epidermis to serve as a sense organ for perceiving the contact stimulus are described.

2. Living motor tissues similar in structure to those described by Haberlandt (2), are to be observed in *Passiflora* and *Luffa*.

3. Different modes of distribution of the mechanical elements in climbing organs are described. The disposition of the mechanical tissues is correlated with the symmetry of the organ. In radially symmetrical organs, such as

twining stems and some tendrils, there is a centripetal tendency in the arrangement of the mechanical tissue. In bilateral organs the mechanical elements are found on the concave and convex sides, those on the concave side being greater in amount than on the convex side.

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EXPLANATION OF PLATE XXIX.

Illustrating the paper by Miss P. M. Kanga and Mr. R. N. Dastur on the Physiological Anatomy of the Irritable Organs of some Climbing Plants.

Fig. 1. An epidermal cell of the stem of *Thunbergia fragrans*, Roxb., in transverse section, showing epidermal ridges, R. × 1,086.

Fig. 2. Epidermis of *Thunbergia alata*, Boj., showing ridges, R, in surface view. × 793.

Fig. 3. Transverse section of a straight tendril of *Vitis quadrangularis*, Wall., showing the corrugated epidermis. × 595.

Fig. 4. Transverse section of a tendril of *Vitis discolor*, Dalz., showing dome-shaped projections, D, of the outer epidermal walls. × 796.

Fig. 5. Transverse section of a tendril of *Passiflora Munroii*, showing the rough epidermis, E. × 864.

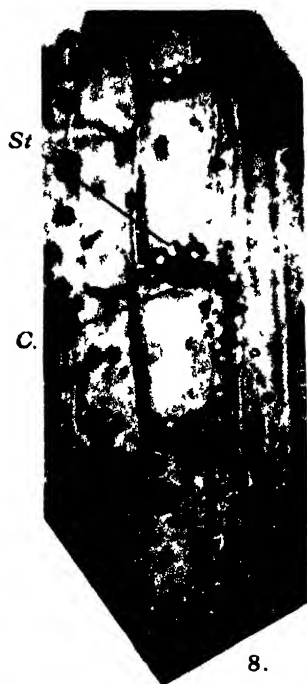
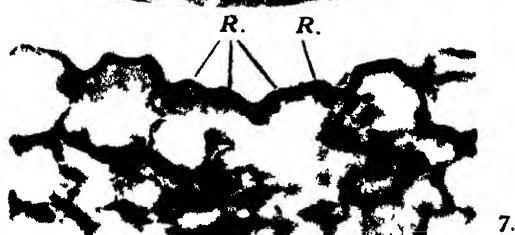
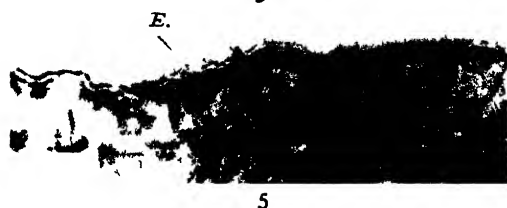
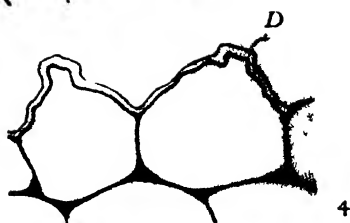
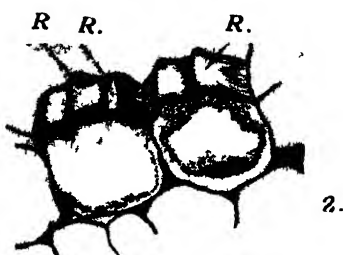
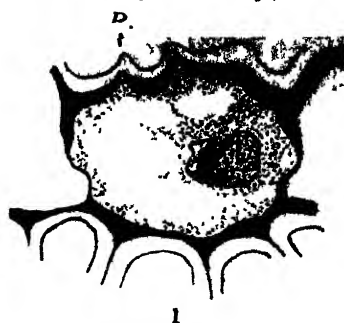
Fig. 6. Transverse section of a tendril of *Vitis latifolia*, Roxb., showing epidermal corrugations, A. × 576.

Fig. 7. Transverse section of the stem of *Thunbergia mysorensis*, T. Anders, showing the epidermal ridges filled with protoplasm. × 704.

Fig. 8. Longitudinal section of the stem of *Thunbergia alata*, Boj., showing the endodermal cells with starch grains, st., and Casparian band, C. × 576.

Fig. 9. Longitudinal section of the stem of *Thunbergia fragrans*, Roxb., showing the transverse oval pits in the cells of the cortex. × 464.

Fig. 10. Longitudinal section of a young and uncurled tendril of *Luffa aegyptiaca*, Mill. a, tactile pits in the outer epidermal walls; b, five tactile pits in a projection of the outer epidermal wall. × 929.



Chromosome Number and the Relationship of Species in the Genus *Viola*.

BY

J. CLAUSEN,

Genetic Laboratory of the Royal Veterinary and Agricultural College, Copenhagen.

With eighty-two Figures in the Text.

INTRODUCTION.

FOR some years past I have been engaged upon cytological and genetic researches within the genus *Viola*, especially as regards the *Melanium* section, and have, in a series of works (12–15), dealt with the two Danish *Melanium* species, *Viola tricolor*, L., and *V. arvensis*, Murr. The two first-mentioned works approach the subject from floristic-statistical, oecological, and taxonomical points of view; in the two last I have subjected the two species to genetic analysis, and compared the genetic process with the cytological conditions found in the hybrids. In the present paper I propose to describe a series of chromosome-number investigations with various species belonging to the genus *Viola*, and to discuss the points arising in connexion therewith. Some of the numbers here given were published by me in 1926, but in the briefest form, without illustrations or further remarks. Though embracing but a relatively small number of the many species—some 500 to 800—included in the genus *Viola*, they nevertheless afford, in conjunction with the researches of other writers (27, 48, 50), a general view from which we can begin to form some idea as to the position of chromosome relationship compared with that based on morphological investigations alone, at any rate as regards the European *Viola* species.

TECHNICAL METHODS.

The cytological investigation was made, in practically every case, from the pollen mother-cells, and the chromosome number determined either from the heterotypic division or from the homotypic metaphase. The

diakinesis is, as a rule, not a suitable stage for the purpose in *Viola*, the conjugation between the chromosomes being here often incomplete. Fixing was formerly effected solely with Carnoy's liquid; this is, however, not particularly suitable when dealing with *Viola* hybrids, as the pollen mother-cells in these are frequently very poor in plasma, and therefore liable to contract too much under the influence of this powerful fixative. The staining then often gives 'black' pollen mother-cells, from which the stain cannot afterwards be removed, unless a small cap has been cut from the cell. Moreover, these cells often become so shrunk and distorted in the fixing that the chromosomes are thrown altogether out of their natural position, and it is then impossible to form any certain conclusions as to the conjugation. In 1926 I used, for hybrids and plants suspected of being such, Navashin's modified fixation fluid after the formula given by Karpechenko (35, p. 367). This I found very successful. It is used as follows:

100 c.c. 1 per cent. chromic acid (CrO_3)	} 11 parts	} mixed together
10 c.c. glacial acetic acid		
16 per cent. formaldehyde (commercial)	} 4 parts	} immediately before use.
formalin 40 per cent.)		

This has proved to be an excellent fixing medium. Even pollen mother-cells greatly deficient in plasma do not shrink.

The flower-buds are of very different sizes when reduction division is in progress. In some types of *V. Kitaibeliana* they are only $\frac{1}{2}$ mm. diameter at this stage, whereas in *V. elatior* they are about 3–4 mm. Cleistogamous flowers reduce at a very early stage, and in these only the extreme outermost portion of the stamen is formed into a pollen sac, so that there are often only three or four pollen mother-cells in one sac. It is therefore best to use the chasmogamous flowers for determination of chromosome numbers.

Staining was formerly effected mainly by means of Delafield's haematoxylin; in dealing with the *Nominium* section, however, this method is of no use. For the past two years I have used the iodine-gentian violet method a great deal, and with excellent results. Specimens of the *Nominium* section, however, should not as a rule be treated for more than ten to fifteen minutes with the 1 per cent. iodine-potassium iodide solution, which is used as a mordant; otherwise, it is impossible to get the chromosomes to stand out dark against an almost colourless background. Certain types cannot be stained at all by the iodine-gentian violet method, as the colour disappears with equal rapidity from chromosomes and plasma alike. Heidenhain's iron-haematoxylin nearly always gives good results, but in the case of certain hybrids, where extranuclear nucleoli appear, it may be risky to use it unless this source of error is borne in mind throughout. Delafield's method is useful here as a check.

CHROMOSOME NUMBERS OF THE SPECIES INVESTIGATED.

In this general survey I shall follow Gingins's (22) old method of division, which appears to be very natural as regards the European *Viola* species, with which I am specially concerned. I have investigated representatives of four of the sections, viz. *Nominium*, *Dischidium*, *Chamaemelanium*, and *Melanium*. Both in the *Nominium* and in the *Melanium* sections we find two systems of chromosome numbers: in *Nominium* a 10-series and a 12-series, in *Melanium* a 10-series and a 6-series. There are, however, a few exceptions.

THE *NOMINIUM* SECTION.*The 10-series.*

V. mirabilis, L., $n = 10$. Examined in the heterotypic anaphase and homotypic metaphase (Fig. 1). Fixed from plants found growing spontaneously in Boserup Woods, Seeland.

V. silvestris, Rchb., $n = 10$. Examined in the heterotypic metaphase (Figs. 2-3). The divisions proceed with the greatest regularity. The type is found growing spontaneously in the Botanical Gardens, Copenhagen, and is altogether identical with the typical *V. silvestris* of the Danish woods.

V. Riviniana, Rchb., $n = 20$. Diakinesis, heterotypic and homotypic metaphase examined. Only the heterotype could be counted with certainty; this, however, was very clear. The chromosomes lie, as in *silvestris*, far apart (Fig. 4). It was remarkable that the two partners forming the heterotypic double chromosome were so closely fused that their double character was only discernible with difficulty (Fig. 5). Material from beech woods in Seeland (Jonstrup Vang).

V. Riviniana \times *V. silvestris*. Hybrids between *Riviniana* and *silvestris* are well known; owing to the numerous intermediate forms, there has been a tendency at times to regard the whole group as one species (*silvatica*, Fries); Linnaeus, indeed, did not distinguish them from *canina*. The unmixed types are, however, the more common in the Danish woods, and they are easily distinguished one from the other, *silvestris* being a more tiny type with very narrow, reddish-violet petals and a thin, tubular, somewhat pointed violet spur, whereas *Riviniana*, as the tetraploid type, is of stronger build, flowers later, and has much broader, bluish-violet petals and a very thick, white spur with blunt end.

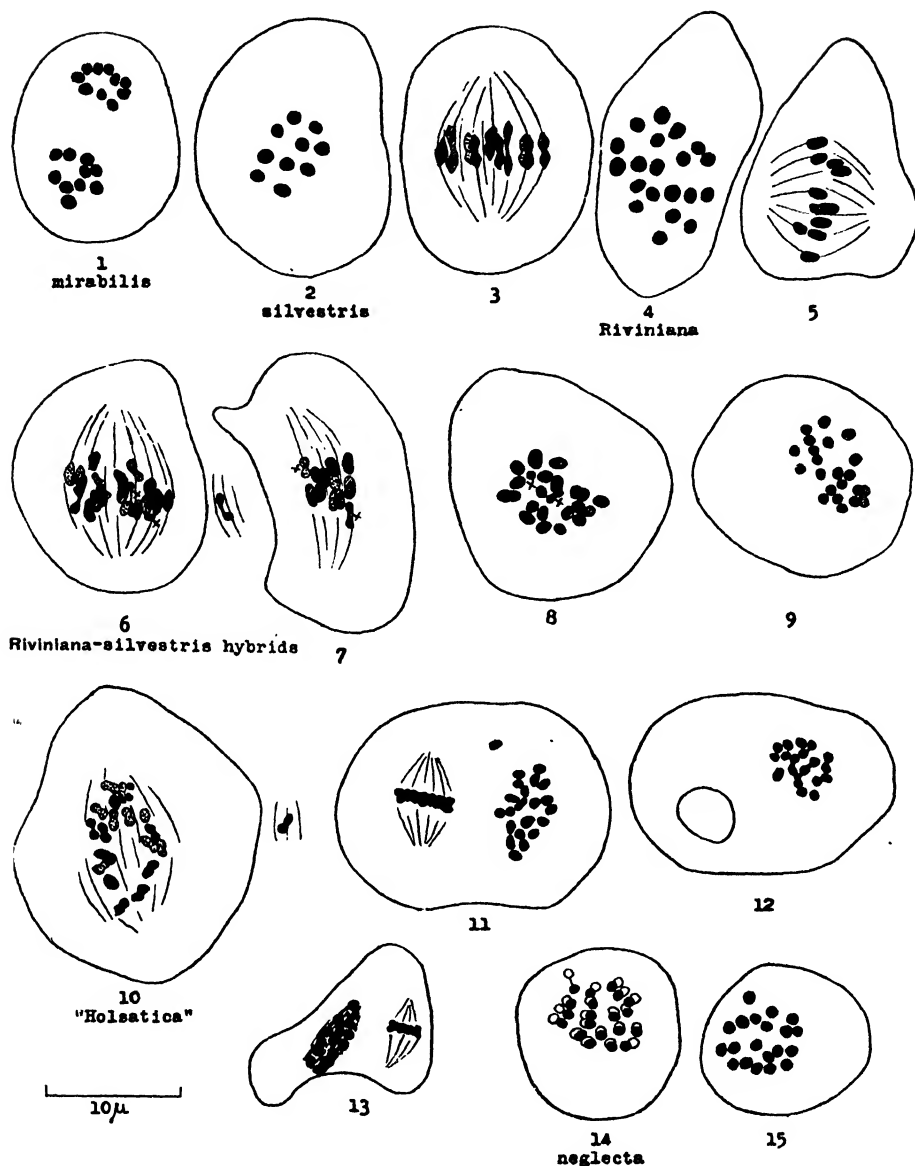
We often find, however, in the Danish woods, types which cannot be referred with certainty either to *silvestris* or to *Riviniana*, the petals, for instance, answering in shape and size to those of *Riviniana*, but in colour to those of *silvestris*. Other types must be classed as intermediate forms both in respect of shape and colour of the flowers. These are, however, not of so frequent occurrence as the pure species.

The cytological investigation shows that the intermediate types have a more or less irregular reduction division, but the chromosome number of the four types which I succeeded in fixing at the right stage answered rather to that of *Riviniana*. There were no F_1 hybrids, and none with chromosome number approaching that of *silvestris*. Two have univalent chromosomes which split in the heterotypic metaphase; in another, the irregularity appears in the fact that there is not such complete order in the conjugation of the chromosomes as in the pure species. Figs. 6-9 show the heterotypic division in three of these types; in Fig. 6 two to three univalent chromosomes will be found in division, in Figs. 7-8 likewise. Fig. 9 is apparently more regular, but two of the chromosomes—drawn lighter here—are possibly univalent, and in other metaphases of the same plant there is more disorder, while the diakinesis often has two to four univalent, some of which may show a tendency to split.

The so-called *V. Riviniana* var. *Holsatica* (Krause), which has at times been regarded as an independent species (*V. Holsatica*, Krause), has also proved, on cytological investigation, to be a hybrid. It differs from *V. Riviniana*, *inter alia*, by the pronounced hairiness of the flower stem, thus approaching *V. rupestris*, Schmidt. The type I had for examination was raised from seed brought from Sonderjylland by Hr. K. Wiinstedt. I wish to express my thanks to Hr. Wiinstedt for his kindness in passing this material on to me for investigation. The flowers resembled those of *Riviniana*, but were much larger, so that I was rather inclined to expect a tetraploid *Riviniana*. Cytological investigation showed, however, that the haploid chromosome number was about twenty (Figs. 10-12), but chromosomes were eliminated from time to time (Fig. 11), and many of the pollen mother-cells were deficient in plasma and highly contracted (Fig. 13). From the results of this investigation, '*V. Holsatica*' must be regarded as a hybrid between *Riviniana* and another species; which, it is difficult to say without a first generation to work on. There may, of course, have been self-pollinations and back-crossings, and types very similar one to the other may perhaps also have been produced by different crossings. The hairy stem does not afford any certain ground for determining which is the other parent, as this character may quite possibly be the result of a new combination of complementary genes; or possibly, again, be due to the elimination of a particular chromosome with an inhibiting gene for hairy stem.

V. neglecta, M. Bieb., $n = 20$. The count was made from the heterotypic metaphase. The chromosomes are nicely bivalent, but considerably smaller than those of *Riviniana* (Figs. 14-15). This type, belonging to Caucasia and Asia Minor, was procured from the Botanical Gardens at Tiflis, and fixed in the Botanical Gardens at Copenhagen.

V. stagnina, Kit., $n = 10$. The count was made from the homotypic metaphase (Fig. 16). This was the Danish type, that grows in the Botanical



FIGS. 1-15. *Nominium* Section, group *Rostrata*. ♀. (*V. mirabilis* and *Rosulantes*). (het. = heterotypic; hom. = homotypic; all figures from pollen mother-cells.) Magnification about 900 times.

FIG. 1. *V. mirabilis*, hom. metaphase. 2-3. *V. silvestris*, het. metaphase; 2 in polar view, 3 in side view. 4-5. *V. Riviniana*, het. metaphase; 4 in polar view, 5 in side view (not complete). 6-9. Spontaneous *V. Riviniana-silvestris* hybrids het. metaphases (univalent chromosomes splitting are marked x); 6-7 in side view, 8-9 in polar view. 7 and 8 belong to the same type; in 6 about 20 and in 8 about 23 units, in 9 20 units: the two shaded ones are possibly univalent. 10-13. '*V. Holsatica*', a spontaneous *Riviniana* hybrid, $n \approx$ about 20. 10, Early het. metaphase in side view; 11-13, hom. metaphases, in 11 one chromosome detached; 13 has shrunk (poor in plasma). 14-15. *V. neglecta*, het. metaphases in polar view.

Gardens at Copenhagen. Heilborn (27) has also investigated *V. stagnina*, and found $n =$ probably 10.

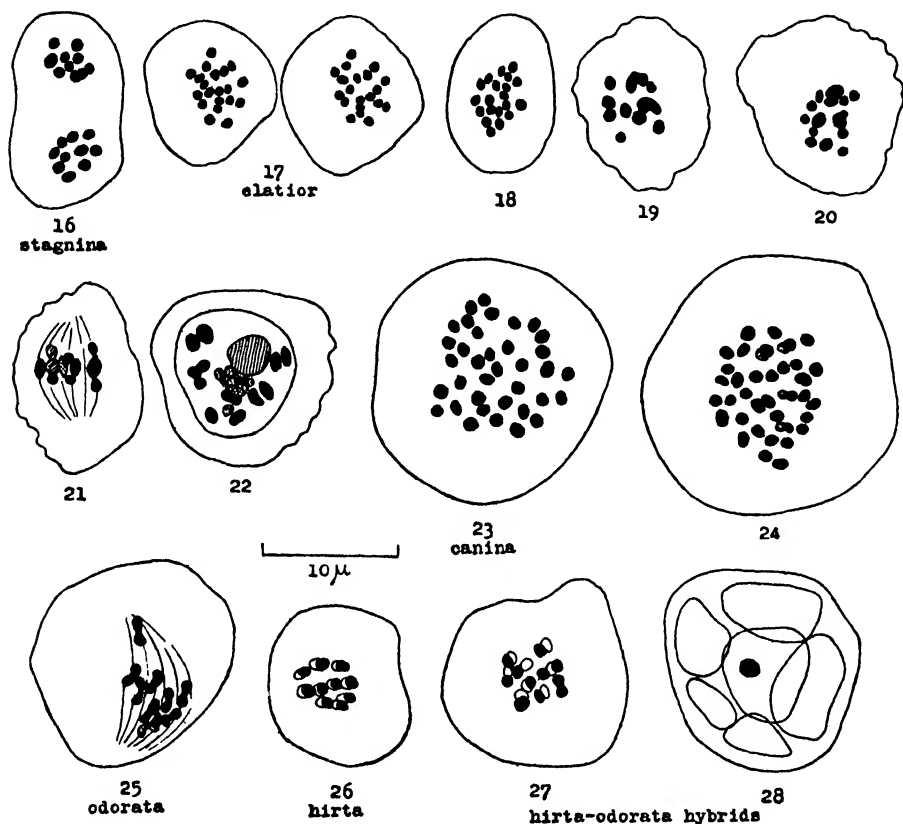
V. elatior, Fries. This species resembles most of all a magnified *V. stagnina*. All the parts are larger. Otherwise, there is but little difference between this and the *stagnina* type investigated, save that *stagnina* is bluish green, whereas *elatior* is of a fresh green colour. The fixed material was obtained from seed sent from the Botanical Gardens at Zürich. The pollen mother-cells shrink very much when the Carnoy method of fixing is employed, and it is difficult to get satisfactory pictures of the reduction division. In a special compartment of a pollen sac, however, some pollen mother-cells were found presenting a fairly clear view; viz. $n =$ about 19 to 20, presumably 20, which would make the species tetraploid in relation to *stagnina* (Figs. 17–18). The chromosomes and pollen mother-cells are very small, far smaller than in *stagnina*, and seem to be altogether poorly developed.

Most of the pollen mother-cells, on the other hand, did not show so many chromosomes in the heterotype; some having no more than about ten, these being then rather larger (Fig. 19); the majority had rather more than ten chromosomes, but the chromosomes themselves were in these cases very unequal in size. Fig. 20 shows one of these pollen mother-cells in which about fourteen units can be counted, six of very large to medium size, and eight small. In side view the largest chromosomes present at times a peculiar appearance. Some are divided into three, as in Fig. 21, the middle portion being the largest, so that one is inclined to wonder whether perhaps the middle portion answers to an ordinary pair of bivalent chromosomes, with two single chromosomes attached, one to each pole of the bivalent. In such case, some of the chromosomes should be quadrivalent. In the diakinesis, Fig. 22, there are about ten gemini, but each of the partners is as large as two of the chromosomes in Figs. 17–18. This also suggests that the seeming gemini here consist each of two gemini, and are thus in reality quadrivalent.

Something similar has been found in the case of *Empetrum hermaphroditum* (24). Just as *V. elatior* is a tetraploid *stagnina*, so also *Empetrum hermaphroditum* ($n = 26$) is a tetraploid *E. nigrum* ($n = 13$). In *Empetrum hermaphroditum* the bivalent chromosomes (including the two pairs of sex chromosomes) are inclined to combine in pairs, two and two, as tetrasomes (cf. the tetrasomic *Datura* (9)). *V. elatior* and *Empetrum nigrum* are doubtless tetraploid races of diploid species formed by doubling of the chromosome number in a relatively homozygous type, where the chromosomes already stand two and two. I have in a previous work (15, p. 135) discussed at some length this form of polyploidy, which Kihara (38) has termed *autopolyploidy* in contradistinction to *allopolyloidy*, which is produced by the doubling of all chromosomes in a specific hybrid.

V. canina, Rchb., $n = 36$. This species forms an exception to the

system otherwise found in the chromosome numbers of all the axilliflorous *Viola* species. For accurate determination of such a high chromosome number, nuclear plates of unusual regularity and clearness are required.



FIGS. 16-28. *Rostratae* continued (group *Arosulantes*) + *Uncinatae*.

FIG. 16. *V. stagnina*, hom. metaphase. 17-22. *V. elatior*. 17, het. anaphase; 18, het. metaphase, 20 bivalent chromosomes (small); 19, het. metaphase, about 10 units (larger); 20, het. metaphase, about 14 units of different size; 21, het. metaphase in side view with tripartite chromosomes (quadrivalents?); 22, diakinesis, about 10 large 'gemini' (quadrivalents?). 23-24. *V. canina*, het. metaphases in polar view. 25. *V. odorata*, transition stage from diakinesis to het. metaphase. 26. *V. hirta*, het. metaphase. 27-28. Spontaneous *V. hirta-odorata* hybrid; 27, het. metaphase in polar view, chromosomes not regularly conjugated; 28, hexad.

Fig. 23 shows a clear and regular heterotypic metaphase of this sort, but the knife has passed just above the nuclear plate, though hardly in such a manner as to remove any chromosomes. Fig. 24 is from a perfectly intact pollen mother-cell, but is, on the other hand, not shown precisely in polar view, and a few of the bivalents can thus appear as two chromosomes. The most likely figure for this nuclear plate, however, is also thirty-six chromosomes.

It was surprising to find such a chromosome number here. The type under investigation was an altogether typical *V. canina*, found growing on a mound of earth between two fields near Lyngby, in Seeland. No other *Nominium* *Violas* were found in the vicinity. The reduction division proceeds very regularly, and, as far as can be seen, all the chromosomes are bivalent. In the early metaphase they are very closely conjugated, just as in *Riviniana*. The type is one that found its way into the experimental grounds at Lyngby, and has been under observation for five years. There is nothing to suggest that it is a hybrid. I have placed it here, as its whole appearance, in spite of all, places it among the *Violas* of the 10-series, and there is, of course, nothing to prevent the secondary formation, by chromosome elimination, of 6-series *Violas* from species belonging to the 10-series. It will be interesting to ascertain whether all *canina* have $n = 36$. It is difficult to obtain a sufficient number of buds at a suitable stage for fixation, when they have to be taken from single plants.

V. odorata, L., $n = 10$. This number has already been published (12) from material collected by Winge. Heilborn (27) has confirmed it. Fig. 25 shows an early heterotypic metaphase with ten bivalent chromosomes. They are not yet arranged in a single plane.

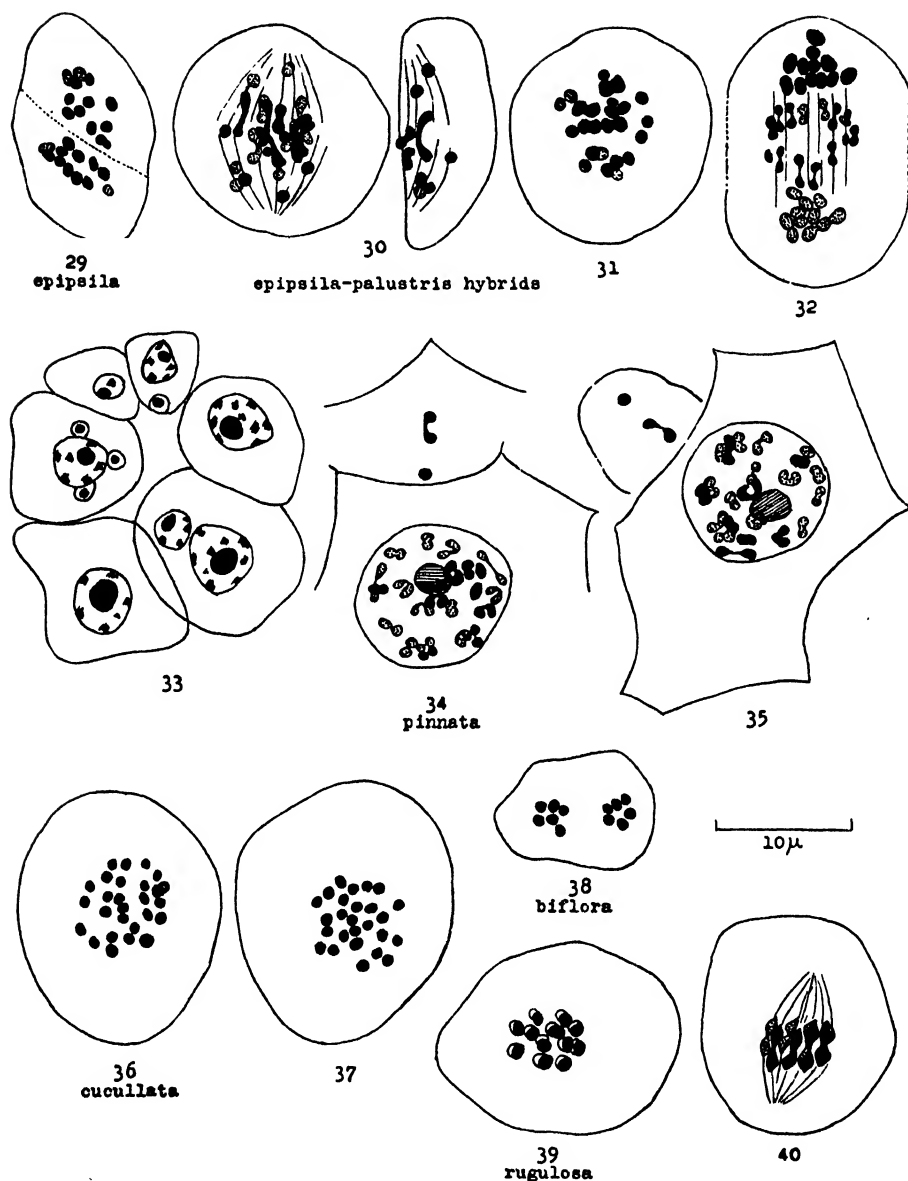
V. hirta, L., $n = 10$. Determined by counting from the heterotypic metaphase (Fig. 26). In 1926 I noted briefly $n = 10$, and this figure was confirmed by Heilborn later in the same year.

A hybrid (presumably *V. hirta* \times *V. odorata*). On one of the lawns in the Copenhagen Botanical Gardens there grows an assortment of acaulescent *Violas*, scented and unscented, with or without short stolons, flowering in many shades of violet. They seem to be intermediate types between *odorata* and *hirta*, and are doubtless segregation products from crossings. True, a cytological investigation showed that the chromosome number was about 10 haploid, but the conjugation was not quite regular (Fig. 27), and pollen formation produced both pentads and hexads (Fig. 28). Schnarf (60) found $n = 10$ for the hybrid between *hirta* and *odorata*. Heilborn (27) has also investigated a hybrid *odorata* \times *hirta*, but he finds both bivalent and univalent chromosomes, which split in the anaphase.

The 12-series:

V. epipsila, Ledeb., $n = 12$. The determination was made from an early homotypic metaphase. The material was procured from a meadow near the lake at Lyngby, Seeland, and was typical *epipsila* (Fig. 29).

V. epipsila, Ledeb. \times *V. palustris*, L., $2n =$ about $12_{II} + 12_I$. In a patch of meadow in a wood in the north of Seeland (Jonstrup Vang) I found a type of *Viola* which I determined as *V. palustris*, though it was not altogether typical. Closer investigation on the spot a couple of years later showed that the types growing in this meadow exhibited different combinations of



FIGS. 29-40. *Nominium* Section, groups *Stolonosae*, *Adnatae*, and *Boreali-Americanae* + Sections *Dischidium* and *Chamaemelanium* (6-series).

FIG. 29. *V. epipsila*, transition stage from interkinesis to hom. metaphase. 30-3. *V. epipsila-palustris* hybrids; 31-2 are from same flower and 30 from another plant; 30, het. metaphase in side view. 9-11 bivalents + 19-21 univalents, of which some are splitting; 31, het. metaphase in polar view, about 12 bivalents (dark) and about 11 univalents (shaded); 32, het. anaphase in obliquely polar view, about 11-12 undivided chromosomes at each of the two poles and about 13 univalent chromosomes dividing in the equatorial plane; 33, pollen hexad. 34-5. *V. pinnata*, diakinesis. 36-7. *V. cucullata*, het. metaphases. 38. *V. biflora*, hom. metaphases. 39-40. *V. rugulosa*, het. metaphases, 39 in polar and 40 in side view (not complete).

those characters which otherwise distinguish *epipsila* from *palustris*. The differences between the two species are, indeed, but slight. Microscopical investigation showed as clearly as could be wished that I was dealing with a hybrid type, as a great number of univalent chromosomes appeared in the heterotypic metaphase. Fig. 30 shows one of these in side view, with presumably 9-11 bivalent + 19-21 univalent chromosomes, 3 or 4 of which are in incipient division. The univalents have not yet arranged themselves in the equatorial plane, but lie scattered about the spindle, while the bivalents are already in the anaphase stage. This gives $2n =$ about 40, but we have here possibly a later generation than F_1 . Fig. 31 shows another heterotypic metaphase in polar view; this is from another flower, and doubtless also from another plant, as it shows about 12 bivalents (dark) + about 11 univalents (light). Fig. 32 shows the anaphase or telophase of the same flower as Fig. 31. At each of the poles will be seen about 11-12 large, undivided chromosomes, while in the equatorial plane there are still 26 half-chromosomes, produced by the splitting of 13 univalents. They are not all equally advanced in division. Fig. 33 shows a pollen hexad of the hybrid. Some of the pollen has several nuclei.

There can be no question of other parents in this case than *epipsila* and *palustris*, and since *epipsila* has 12 chromosomes, *palustris* must presumably have 24. I fixed a great deal of material from a pure population of *V. palustris*, but did not succeed in getting stages suitable for counting. Over 20 chromosomes could, however, be discerned.

Doubt has frequently been expressed as to the value of *V. epipsila* as a species. The hybrid here in question proves that *epipsila* and *palustris* are distinct species. *V. epipsila* has doubtless the more primitive chromosome number of the two. In all probability the hybrids are of more frequent occurrence than is generally recognized, for with such slight morphological differences one would not often venture to insist that a not altogether relevant type was a hybrid, unless of course on cytological investigation.

V. pinnata, L., $n =$ about 24. Fixed in the Copenhagen Botanical Gardens. I have only seen the diakinesis (Figs. 34-5). The formation of gemini is very good, but there is nevertheless always some uncertainty in counting from the diakinesis, though counts made from several such always gave from 23 to 25 chromosomes, with 24 as the most likely figure.

V. cucullata, Ait., $n = 26$. Just as *tricolor* ($n = 13$) must be regarded as belonging to the 6-series, so also *V. cucullata*, which has 2×13 chromosomes, should be included here. The count was made from the heterotypic metaphase (Figs. 36-7). The conjugation is very complete, and the divisions are very regular. The fixed material is from the Copenhagen Botanical Gardens.

THE *DISCHIDIUM* SECTION.

V. biflora, L., $n = 6$ (Fig. 38). This is the lowest figure I have found in any *Viola*. Miyaji (48) also found six chromosomes in the yellow-flowered *V. glabella*, Nutt., which has some resemblance to *biflora*, but belongs to the *Chamaemelianum* group. In *V. biflora* both the chromosomes and the pollen mother-cells are very small. Material from Copenhagen Botanical Gardens.

THE *CHAMAEMELANIUM* SECTION.

V. rugulosa, Greene, $n = 12$. The species was found growing in the Copenhagen Botanical Gardens, having been received there under the name of *V. Rydbergii*, Greene. Brainerd (11) considers that *Rydbergii* can no longer be maintained. The divisions take place with extreme regularity, as may be seen to some extent from Figs. 39-40.

THE *MELANIUM* SECTION.*The 10-series:*

V. cenisia, L., $n = 10$. The plants were altogether typical, grown from seeds from H. Correvon's alpine garden at Geneva. The count had to be made from the diakinesis, but with the small number of gemini here in question, there can be no uncertainty about it (Fig. 41).

V. elegantula, Schott., $n = 10$. (Syn. *V. latisejala*, Wettst.; *V. bosniaca*, Formanek.) Grown from seeds received from the Gothenburg Botanical Gardens, under the name of *bosniaca*. Shows very handsome and regular heterotypic division (Fig. 42).

V. declinata, Waldst. et Kit., $n = 10$. Grown from seed supplied by the 'Fredow' arboretum near Leopold, Poland. Typical *Viola declinata*. The divisions were regular (Figs. 43-5).

'*V. Valderia*,' $n = 10$. The seeds were received from H. Correvon under this name, and had been gathered wild in the Alpes Maritimes. The leaves were distinctly crenate, so it was not *V. Valderia*, All., which is closely related to *cenisea*. The plant actually corresponded in every respect to *V. Valderia*, Rchb., which is generally referred to *V. heterophylla*, Bertol. It was one of the western alpine types, which form the transition from *V. declinata* via *V. Dubyana*, Burn., to *V. heterophylla*, Bertol., and thence to *V. calcarata*, L. The chromosome number was determined from a transition stage between the interkinesis and the homotypic metaphase (Fig. 46).

V. cornuta, L., $n = 11$. The divisions proceed with perfect regularity, and there are 11 bivalent chromosomes in the heterotypic division. Fig. 47 shows a heterotypic anaphase. The plant was grown in the Copenhagen Botanical Gardens, and was a typical *cornuta*, L.

Heilborn (27), on the other hand, gives 10 as the most likely figure, but notes that univalents occur, splitting longitudinally, and considers that there are 9 bivalents and 2 univalents. Heilborn's Fig. 1, *f*, undoubtedly shows 10 chromosomes at the one pole and 11 at the other, but he considers that two of these (marked \times) are half-chromosomes produced by longitudinal splitting. The two chromosomes marked \times are, however, surely too large to be regarded as halves, and I have no doubt but that Heilborn's specimen was a hybrid, e.g. between *V. cornuta* and *V. elegantula*. Wittrock (69) has already described and figured this hybrid form (Pl. VII, Figs. 89-92; Pl. IX, Figs. 125-31); and I once received myself, from the Botanical Gardens at Lausanne, seeds of *V. latisepala*, Wettst. (= *elegantula*, Schott.), from which there likewise grew a couple of hybrids between *elegantula* and *cornuta*; these exhibited corresponding irregularities. The hybrid closely resembles *V. cornuta*, and when found in a *cornuta* population would probably pass unnoticed, unless one happened to know it exceptionally well.

V. cornuta, L. \times *V. elegantula*, Schott. For comparison with Heilborn's figure I give here a homotypic metaphase of an authentic F_1 of *cornuta* \times *elegantula*, produced by crossing (Fig. 48). The heterotypic division presents a somewhat irregular appearance, but in the homotypic metaphase we find either 10 or 11 chromosomes in the nuclear plates; I have never seen chromosomes detached. There must, then, presumably be complete conjugation between the 10 chromosomes of *elegantula* and the 10 of *cornuta*, the eleventh *cornuta* chromosome being distributed haphazard, i. e. after the *Drosera* scheme. Out of 19 homotypic metaphase plates found suitable for counting, 10 had 11 chromosomes, and 9 had 10. The hybrid is, however, almost sterile; it forms, at any rate, but very few seeds on self-pollination.

V. orthoceras, Ledeb., $n = 11$. The fixed plant was grown from seeds furnished by Dr. G. Voronov, of the Tiflis Botanical Gardens. Up to now, there has been but little material available. Staining with iodine-gentian violet proved unsuccessful; Heidenhain's method was better. The homotypic metaphase, however, undoubtedly exhibits 11 chromosomes (Figs. 49-50).

It is very interesting to note that *cornuta* and *orthoceras*, which are so much alike that they have been regarded at times as belonging to the same species, both have 11 chromosomes, the more so since they are highly localized and widely separated in geographical respects. *Viola cornuta* is restricted to a small area in the Pyrenees, *orthoceras* to a corresponding area in the Caucasus and Armenia (4, 7). There are, however, in the Balkan Peninsula, a few species apparently occupying intermediate positions between *Viola elegantula* and the two here named. This applies especially to:

V. Orphanidis, Boiss. I have had some plants under cultivation which I had received from various botanical gardens under this name.

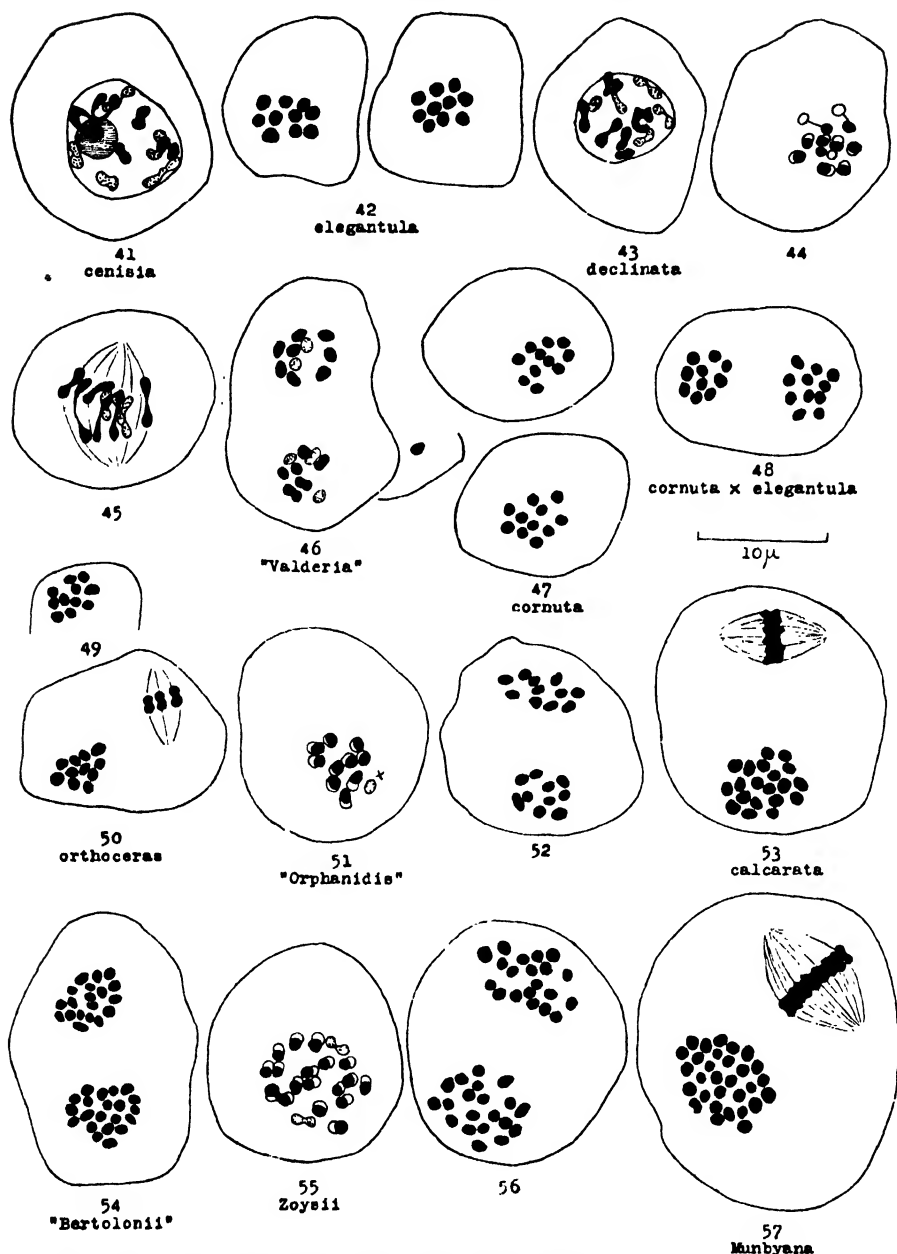
FIGS. 41-57. Section *Melanium*, group *Calcaratae* (10-series).

FIG. 41. *V. cenisia*, diakinesis. 42. *V. elegantula*, het. anaphase. 43-5. *V. declinata*; 43, diakinesis; 44-5, het. meta-anaphase, 44 in polar, 45 in side view (not complete). 46. '*V. Valderia*', transition stage between interkinesis and hom. metaphase. 47. *V. cornuta*, het. anaphase. 48. *V. cornuta* x *elegantula* F_1 , hom. metaphase, 10 + 11 chromosomes. 49-50 *V. orthoceras*, hom. metaphases. 51-2. '*V. Orphanidis*', a plant with 10 bivalents and 1 univalent (x) chromosome; 51, het. metaphase; 52, hom. metaphase, 10 + 11 chromosomes. 53. *V. calcarata*, hom. metaphase. 54. '*V. Bertolonii*' (a *calcarata* type), hom. metaphase. 55-6. *V. Zoisii*; 55, het. metaphase in polar view, two bivalents lying horizontally; 56, hom. metaphase. 57. *V. Munbyana*, var. *Battandieri*, hom. metaphase.

They agree also with the diagnosis and extant figures, but have doubtless nevertheless been crossed with other species of the group, being irregular in the reduction division. Figs. 51–2 show the heterotypic metaphase with $10_{II} + 1_I$, and a pair of homotypic metaphases, one with 10, the other with 11 chromosomes. It is thus impossible to determine, from this material, whether *V. Orphanidis* agrees in chromosome number with *V. cornuta-orthoceras* or with *V. elegantula*.

I have included *V. cornuta* and *orthoceras* in the 10-series, though they do not fully fit in here, and despite the fact that it is impossible to determine whether they are derivatives of a 10-chromosomed or of a 12-chromosomed type. But their whole appearance ranks them most nearly among species of the 10-series such as *V. elegantula* and *V. calcarata*. Moreover, they appear to be most easily crossed with species belonging to the 10-series.

V. calcarata, L., $n = 20$. The plants were grown from seeds supplied by H. Correvon, of Geneva. They did not attain full bloom, but the vegetative parts agreed well with *V. calcarata*. There did not appear to be any irregularity in the divisions (Fig. 53). Another *calcarata* type, received from the Botanical Gardens at Upsala under the name of *V. Bertolonii*, Salis. (= *corsica*, Rouy et Fouc.), had $2n = 40$, but cases of non-disjunction occurred here (Fig. 54), 21 chromosomes going to the one pole, and 19 to the other. In morphological respects it was a fairly typical *V. calcarata*, save that the spur was rather short.

V. Zoysii, Wulf., $n = 20$. The plant used was supplied to the Copenhagen Botanical Gardens from Sündermann's alpine garden at Lindau, near the Bodensee. It was a typical *Viola Zoysii* (not *V. calcarata*, L., var. *flava*, Gren. et Godr.). The heterotypic metaphase occasionally showed gemini parallel to the equatorial plane, not at right angles to it (Fig. 55). This gave rise now and then to non-disjunction, the homotypic daughter nuclei containing $19 + 21$ chromosomes. Fig. 56 shows a pair of homotypic daughter nuclear plates, each with 20 chromosomes. The cytological features are otherwise entirely in accord with those of *V. calcarata*, so that there is no criterion here for distinction between them as species.

V. Munbyana, Boiss. et Reut., var. *Battandierii* (W. Beckr. pro spec.), $n = 30$ (Fig. 57). The plants were grown from seeds received under the name of *V. gracilis*, Sibth. et Sm., from the Muséum d'Histoire naturelle in Paris. They were completely identical with the *Viola Munbyana* shown in Wittrock (69, Pl. XI, Figs. 173–7). The type in question must evidently be a variety known only from botanical gardens. It is received under various names, and must be regarded as a kind of *varietas hortensis*; I have, however, in the herbarium of the Copenhagen Botanical Museum, seen specimens from Temiât-el-Had, in Algeria, very much resembling the type

from the Botanical Gardens. These specimens were collected at intervals of several years by C. M. Poulsen and A. Letourneux. The variety investigated is doubtless identical with the type which Kristofferson (40) crossed with *V. tricolor*. The type I have employed is also very easy to cross both with *V. tricolor* and *V. lutea*, Huds., but not, on the other hand, with *V. elegantula*, nor did I succeed in producing hybrids from it with *V. cornuta*. These facts might seem to suggest that it should be regarded as a decaploid species of the 6-series, rather than a hexaploid of the 10-series. It is nevertheless very much like *V. calcarata* in appearance, and until the whole section has been more exhaustively crossed than is now the case there is no adequate reason for transferring it to the 6-series.

The 6-series:

V. Kitaibeliana, Roem. et Schult., $n = 7$, about 12, 18. I have several times received seeds from the Tiflis Botanical Gardens under the name of *V. Kitaibeliana*. One of the consignments consisted of a mixture of *V. arvensis* with $n = 17$ (Fig. 67) and a *Kitaibeliana* type with $n = 18$ (Figs. 60-2). Another batch was more uniform in appearance; it was made up exclusively of typical *V. Kitaibeliana*, which I presumed had also $n = 18$, though the type was of rather slighter build than the first. A crossing with *arvensis* was made before I had an opportunity of subjecting the new type to cytological investigation. In the F_1 of this crossing, however, there were about 10 univalents, not, as I had expected, only one. Cytological investigation of the root-tips from the crossed *Kitaibeliana* type showed, to my surprise, that this had $2n = 14$ (Fig. 58). Another plant from the same batch as the last and of altogether the same appearance had $n =$ about 12 (Fig. 59), but as the count was made from the diakinesis, I dare not give this figure with absolute certainty. As mentioned, the 18-chromosomed type is of rather more powerful growth than the two others, but the shape of the flowers is quite typically that of *V. Kitaibeliana*. All these three belong to the very small-flowered type, but several authorities on classification (5, 21) further refer some rather large-flowered types to *V. Kitaibeliana*. It would be interesting to ascertain definitely the chromosome numbers for the whole of this morphological group, comprising *V. Kitaibeliana*, Roem. et Schult. (= *nemausensis*, Jord.), *hymettia*, Boiss. et Heldr. (= *olyssiponensis*, Rouy), *Henriquesii*, Willk., '*tricolor* var. *trimestris*, DC.', *parvula*, Tin., and *Helzreichiana*, Boiss. Possibly yet other different types—as regards the chromosomes—may be isolated from these Tiflis consignments. Unfortunately, I have not gathered any seeds of the 12-chromosomed type. The three types must be regarded as diploid, tetraploid, and hexaploid respectively. The figure 7 is the lowest recorded up to now in the *Melanium* section, and must be regarded as a derivative from a 6-chromosomed type.

V. tricolor, L., sens. strict., $n = 13$. See previous works. Fig. 63 shows homotypic metaphase of an extreme type: var. *maritima emarginata*. This type is growing on the island Læsø in Kattegat (13, p. 367).

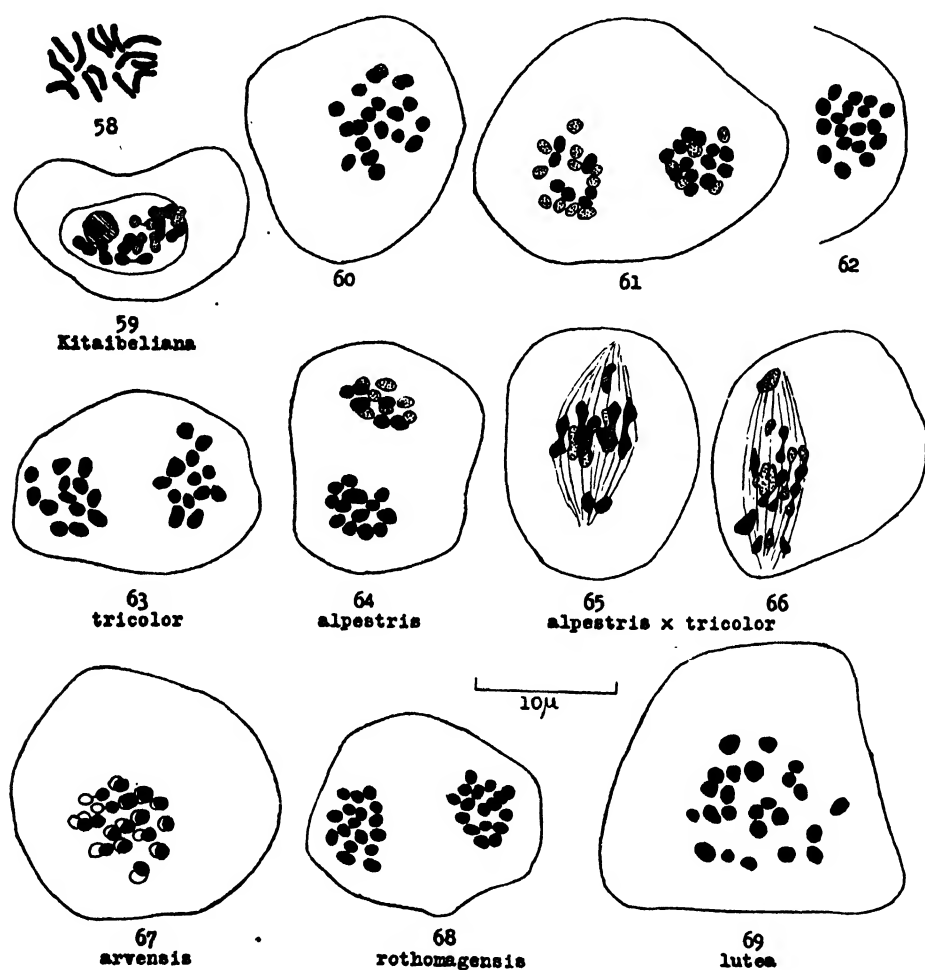
V. alpestris, (DC.) W. Beckr., $n = 13$ (Fig. 64). The type fixed was grown from seed gathered wild at Brno in Czechoslovakia. It does not appear to differ cytologically from *V. tricolor*, nor is there any great difference in morphological respects. *V. alpestris* is a subalpine, 2–4-year type; *tricolor* can also, however, become perennial in particular localities, as among conifers for instance, or in sand dunes (12 and 13). *V. alpestris* has generally yellow flowers, but there are also yellow-flowered varieties of *tricolor*, differing only from the violet in one gene. When *arvensis* is crossed with *tricolor*, these large-flowered and yellow-flowered types are always formed, *arvensis* providing the gene for yellow colouring, which is epistatic to the violet (15). It is not impossible that the *alpestris* types may have been formed by crossing between *arvensis* and *tricolor*.

V. alpestris \times *tricolor*. I have crossed *tricolor* with *alpestris*, but not all *tricolor* chromosomes are capable of conjugating with the corresponding ones of *alpestris* (Figs. 65–6). The univalent chromosomes often have a tendency to split in the course of the heterotypic division, in a similar way to those of the plant V. 209–3 (14 and 15), which was the original mother of *Viola hyperchromatica* ($n = 23$). In one particular F_1 plant of *tricolor* \times *alpestris*, indeed, nearly all the twenty-six chromosomes appeared as univalents, dividing in the heterotypic metaphase. This fact, that the chromosomes of *tricolor* and *alpestris* behave as if they were specifically different, suggests that they should be regarded as distinct species, though it may sometimes be difficult to distinguish one from the other. In any case, they are two genetically distinct ecotypes, viz. an ecotypus *campestris* (*tricolor*) and an ecotypus *subalpinus* (*alpestris*), which are on the verge of being specifically distinct.

V. arvensis, Murr., $n = 17$. Has been previously dealt with together with *tricolor*. Fig. 67 shows heterotypic metaphase of a type from Caucasia.

V. arvensis \times *tricolor*. The artificially produced hybrids between *tricolor* and *arvensis*, and their segregation, were first described by Kristoferson (39, 40); and I have, in my own work of 1926 (15), treated the segregated types in detail. Wittrock (69, pp. 107–15) has described spontaneous hybrids from Gothland. Spontaneous hybrids are of fairly frequent occurrence among the wild plants, though the pure species are, at any rate in Denmark, far more numerous. The pure species grow each on its own type of soil, *tricolor* preferring the acid, *arvensis* the more alkaline type, while the hybrids are found on neutral to slightly acid soil, where both species can grow (13).

Figs. 7-8 in my paper of 1922 (13) show the reduction division in one of these spontaneous hybrids of a later generation than F_1 . It had fourteen



FIGS. 58-69. *Section Melanium*, group *Tricolores* (6-series).

FIG. 58. *V. Kitaibeliana* type, $2n = 14$, somatic plate from root-tip. 59. Another *V. Kitaibeliana* type, diakinesis, $n =$ about 12. 60-2. A third *V. Kitaibeliana* type, $n = 18$; 60, het. metaphase; 61, transition stage between interkinesis and hom. metaphase; 62, hom. metaphase. 63. *V. tricolor maritima emarginata* (Lacsö type), hom. metaphase. 64. *V. alpestris*, early hom. metaphase. 65-6. *V. alpestris* \times *tricolor* F_1 , het. metaphases in side view (not complete), univalent chromosomes undivided and dividing. 67. *V. arvensis*, a type from Caucasia, het. meta-anaphase in polar view. 68. *V. rothomagensis*, hom. metaphase. 69. *V. lutea*, het. metaphase in polar view, 24 bivalent chromosomes.

bivalent and two univalent chromosomes. Fig. 13, Pl. II, in the paper of 1921 (12) is doubtless from a hybrid, though the plant was a typical *arvensis* to look at. Heilborn (27) is doubtless right in suggesting that the four small chromosomes between the anaphase plates are two split univalents; the

chromosomes noted as displaced by cutting, on the other hand, are not nucleoli, as Heilborn appears to think, for the slide was stained with P. Mayer's acetous haemalum, which gives nucleoli a shade of colour different from that of the chromosomes. In the mentioned figure there are twelve and fourteen chromosomes respectively at the poles, and between them two univalents in process of division. I have also received from Riga, in Latvia, under the name of *V. tricolor*, seeds gathered from plants growing wild; these proved, both morphologically and cytologically, to be seeds of hybrids between *tricolor* and *arvensis*.

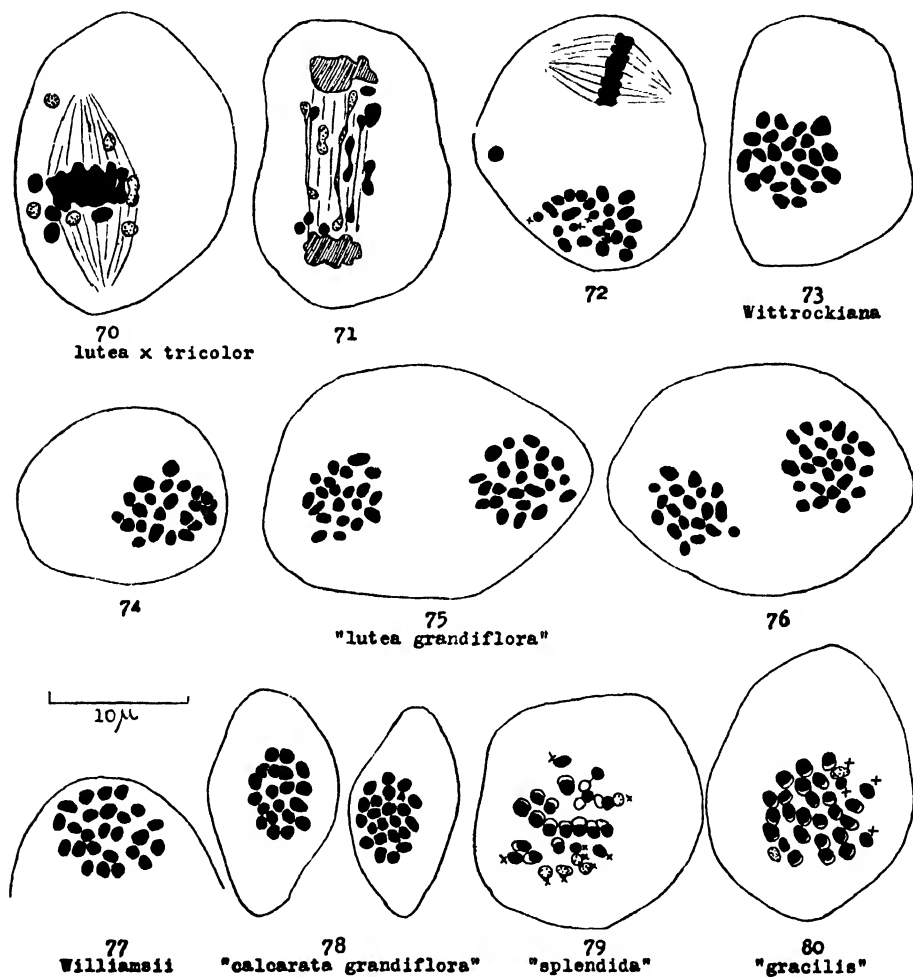
V. rothomagensis, Desf., $n = 17$ (Fig. 68). This species, which has but a very limited area of distribution, being confined to the calcareous district in the neighbourhood of Rouen, bears not the slightest resemblance to *arvensis* in outward appearance, though its chromosome number is the same. It looks, indeed, far more like *tricolor*, having large flowers, violet in colour; it is, however, very hispid, and typically perennial. The plants were grown from seeds supplied by the Botanical Gardens at Rouen.

V. lutea, Huds., $n = 24$ (Fig. 69). The plants investigated were grown from seeds received from the Glasgow Botanical Gardens under the name of *V. tricolor*. They were typical *V. lutea*, Huds., subs. *elegans*, (Kirschl.) W. Beckr., of the characteristic type and with underground stolons. The pollen mother-cells are, as will be seen, very large. The variety *calaminaria*, Lej., has a chromosome number very near to twenty-four, not exactly determined however.

V. lutea, Huds. \times *V. tricolor*, L. According to Wittrock's (68) investigations our cultivated pansies were produced in the 1830's by English gardeners, from this cross. I have crossed the old *V. tricolor hortensis* ($n = 13$) with the wild type of *V. lutea*. Fig. 70 shows the heterotypic metaphase in F_1 of this hybrid. The bivalent chromosomes cannot be distinguished, but there are at any rate 9 univalent. Fig. 71 shows the anaphase. There are 11 univalents visible here; of these, 10 are either already split or in process of splitting, only one (large) remaining undivided. The bivalent chromosomes have already reached the poles, at a somewhat earlier stage, but cannot be counted. Fig. 72 shows a homotypic metaphase with about 24 chromosomes, of which some are only half (univalent, divided) chromosomes; one chromosome is detached.

This process corresponds to that noted in the case of *epipsila* \times *palustris*. As a result, the entire offspring will have 24 chromosomes, or something like it, and we also find that the cultivated Pansies have chromosome numbers in the neighbourhood of 24 (Figs. 73-6). By this process, the *lutea* chromosomes gain the ascendancy in the offspring; and the cultivated pansies are also seen to resemble this form. The name given them in gardening catalogues, *V. tricolor maxima*, is thus misleading. Gams (21) has suggested the name *V. Wittrockiana*, and I agree with this,

inasmuch as they cannot be regarded either as *lutea* or as *tricolor* types, but must be taken as an entirely new species produced by crossing. It has



FIGS. 70-80. *Hybrids and garden types of the Section Melanium.*

FIGS. 70-2. *V. lutea* x *tricolor* F_1 ; 70, het. metaphase in side view, a number of bivalents and at least 9 univalents; 71, het. anaphase in side view, 11 univalents between the two anaphase plates, 1 undivided and 10 in a state of division or already divided; 72, hom. metaphase, 1 chromosome detached, in the nuclear plate about 20 undivided and 3 divided (x) chromosomes. 73-4. *V. Wittrockiana* (= '*tricolor maxima*'); 73, hom. metaphase, 24 chromosomes; 74, het. metaphase, about 23 units. 75-6. '*V. lutea grandiflora*', hom. metaphases; 75, 21 + 23 chromosomes; 76, 19 + 25 chromosomes. 77. *V. Williamsii* (= '*cornuta hybrida*'), nom. metaphase, 25 chromosomes. 78. '*V. calcarata grandiflora*', Correvoon, het. anaphase, 20 + about 22 chromosomes. 79. '*V. splendida*', Correvoon, het. metaphase in polar view, about 16 bivalents + 9 univalents. 80. '*V. gracilis*', Correvoon, het. metaphase in polar view, 24 bivalent + 4 univalent chromosomes.

not proved possible, however, to find a cytologically constant type among the *Wittrockiana*. The plants are constantly crossing, and exchanging chromosomes. And as some of the types will presumably have an extra

large number of the original *tricolor* chromosomes, and fewer in proportion of the *lutea*, while in others the proportion will be reversed, conjugation will often be incomplete; gametes with different numbers of chromosomes will be formed, and the irregularity in the chromosome numbers of the pansies thus maintained. Figs. 75–6 show by way of example the homotypic metaphase of a large, yellow-flowered type ('*V. lutea grandiflora*'). The pollen mother-cell of Fig. 75 has reduced in such unequal fashion as to give 19 chromosomes at one pole and 25 at the other.

The so-called '*V. cornuta hybrida*' (*V. Williamsii*, Wittr.) is, as Wittrock (68) has shown, formed by a cross between Pansies and *V. cornuta*, i. e. about 24 chromosomes \times 11 chromosomes. Most of these types resemble in appearance *V. Wittrockiana*, Gams, especially in the broad type of flower and in the leaf system. From *cornuta*, on the other hand, they inherit their perennial character, and, in the case of several types, their scent. Cytologically, they resemble *Wittrockiana*, not *cornuta*, having about 24 chromosomes. Fig. 77 shows a homotypic metaphase of *V. Williamsii*, Wittr., with 25 chromosomes.

There are, however, also garden hybrids which resemble *V. cornuta*, as for instance the velvety '*Gustav Wermig*' with narrow, deep velvety violet petals. This type is almost sterile. It has about 11 chromosomes in the sex cells, but it is very difficult to investigate, owing to the great irregularity of the reduction division; large portions of the pollen sacs consist of tissue altogether sterile, resembling the tapetal.

Most of the *Melanium* *Violas* from botanical and other gardens are, indeed, very irregular in their reduction division. In the natural state they grow in localities geographically far apart, but as soon as they are brought together in gardens, they take the opportunity of crossing, partly without regard to chromosome numbers. Most of the *Melanium* *Violas* are capable of crossing with one another, though the hybrids for the first few generations are not very fertile. The majority of alpine species thrive but very poorly in ordinary botanical gardens, but crossings—and possibly mutations—lead in course of time to the differentiation of types less specialized than the pure alpine species, and which thrive excellently, though their chromosome features are rather irregular. By way of example, I give here illustrations of the reduction division in three plants from three different types received from H. Correvon, of Geneva. Fig. 78 shows the heterotypic anaphase of a plant of the so-called '*V. calcarata grandiflora*' with 20 chromosomes in the one anaphase plate, and about 22 in the other; $2n$ = about 42. Fig. 79 shows the heterotypic meta-anaphase of a so-called '*V. splendida*', with about 16 bivalent and about 9 univalent chromosomes, $2n$ = about 41; about 20 chromosomes will go to the upper pole, and about 21 to the lower. These two last-named types are very much alike, and doubtless belong to *V. calcarata*, sens. lat. In contrast to the typical

V. calcarata, they thrive excellently everywhere. Fig. 80 shows the heterotypic anaphase of '*V. gracilis*' with about 24 bivalent and 4 univalent chromosomes, $2n =$ about 52. 27 chromosomes will go to the upper pole, and only 25 to the lower. This type differs somewhat from the two last.

DISCUSSION.

Chromosome Numbers and Specific Hybrids.

Some *Viola* hybrids in their reduction division follow the *Drosera* type (56), the univalent chromosomes being distributed haphazard between the two poles without division; thus as a rule *arvensis* \times *tricolor* and



FIG. 81. Diagram representing reduction division in F_1 of a hybrid between two species, one with 20 (black) chromosomes and one with 10 (dotted) chromosomes. Ten bivalents are formed, and the diagram shows a case in which 6 dotted + 4 black chromosomes go to the upper pole, while 4 dotted + 6 black ones go to the lower one. The 10 black univalent chromosomes all split, thus in addition giving 10 black chromosomes to each of the two poles.

cornuta \times *elegantula*. Others follow the *Triticum* type (36, 37), the univalent chromosomes splitting already in the heterotypic metaphase. In *Viola* the chromosomes, like those of *Rosa*, but unlike those of *Triticum*, doubtless also split in the homotypic division. *V. epipsila* \times *palustris*, *lutea* \times *tricolor*, and probably also *Riviniana* \times *silvestris*, behave in this manner.

If there is no elimination of chromosomes, hybrids following the *Drosera* type will exhibit something approaching Mendelian segregation also in regard to those genes associated with the univalent chromosomes. It is otherwise with hybrids following the *Triticum* type. F_2 will, indeed, be homozygous in regard to characters determined by genes associated with the univalent chromosomes. A hybrid between *V. Riviniana* and *V. silvestris*, for instance, will receive 20 chromosomes from the former and 10 from the latter. In F_1 all the 10 *silvestris* chromosomes will conjugate with 10 of the *Riviniana* chromosomes, while 10 univalent *Riviniana* chromosomes will split and distribute themselves equally, 10 halves to each of the two daughter nuclei. The diagram Fig. 81 shows the result of this. The 10 *silvestris* chromosomes are drawn lighter in colour, the 20 *Riviniana* black. The 10 bivalent chromosomes on the left are drawn large, while the 10 univalent on the right are drawn as small chromosomes in process of division. The 10 bivalents consist in every case of a *silvestris* and a *Riviniana* partner, which find their way haphazard to one pole or the other,

and thus will give rise to Mendelian segregation. In the case here considered, we have 6 *silvestris* chromosomes going to the upper pole, with 4 undivided + 10 divided *Riviniana* chromosomes to the same. Four *silvestris* chromosomes, with 6 undivided and 10 split *Riviniana* chromosomes, go to the other side. All the gametes will then contain at least 10 *Riviniana* chromosomes, and occasionally all the chromosomes in a gamete may prove to be *Riviniana*. The greatest number of *silvestris* chromosomes that any gamete can obtain is 10, and this only in rare cases; most frequently there will be 5 *silvestris* and 15 *Riviniana* chromosomes in the gametes. From this it will be seen that in specific hybrids, where the univalent chromosomes split in the heterotypic metaphase, the species with the higher chromosome number will obtain an altogether predominant influence on the appearance of the offspring, provided that the chromosomes in question also split in the homotypic division.

CHROMOSOME NUMBER AND CLASSIFICATION.

At the present we know the chromosome numbers of some forty 'pure' species belonging to the genus *Viola*. Miyaji (48) was the first to investigate *Viola* cytologically, and he found, at that early date, the series 6, 12, 24, 36 in *Viola*, even before Tahara (62) had published the handsome series for *Chrysanthemum*. I give here, in the table, below, all the known chromosome numbers, arranging the species mainly according to the latest system published by W. Becker (8) in Engler and Prantl, 'Natürliche Pflanzenfamilien', and based on many years of close study. Author and reference are supplied for counts other than those given in this paper. The number given is the haploid (n). An asterisk before the number denotes that it is calculated from counts made in the somatic cells.

Chromosome Numbers in *Viola*.

Sect. NOMINIUM.

I. Uncinatae.

	n	FIG.
<i>odorata</i> , L. (Clausen, 12, Heilborn, 27)	10	25
<i>hirta</i> , L. (Clausen, 15, Heilborn, 27)	10	26
<i>nipponica</i> , Maxim. (Miyaji, 48)	10	

II. Rostratae.

<i>mirabilis</i> , L.	10	I
<i>silvestris</i> , Rchb.	10	2-3
<i>grypoceras</i> , Gray (Miyaji, 48)	10	
<i>Riviniana</i> , Rchb.	20	4-5
<i>neglecta</i> , M. Bieb.	20	14-15
<i>stagnina</i> , Kit. (Clausen, 15, Heilborn, 27)	10	16
<i>elatior</i> , Fries.	20	17-22
<i>canina</i> , Rchb.	36	23-24

III. Bilobatae.

<i>verecunda</i> , Gray (= <i>alata</i> , Bürgersd. var.) (Miyaji, 48)	10	
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Chromosome Numbers in *Viola* (continued).

n FIG.

IV. Stolonosae.

<i>epipsila</i> , Ledeb.	12	29
<i>palustris</i> , L. (by calculation from a hybrid)	likely	24	

V. Adnatae.

<i>Okuboii</i> , Makino (= <i>Keiskei</i> , Miq. var.) (Miyaji, 48)	12	
<i>phalacrocarpa</i> , Maxim. (Miyaji, 48)	12	
<i>japonica</i> , Langsd. (Miyaji, 48)	24	
<i>pinnata</i> , L.	abt.	24	34-35
<i>Patrini</i> , L., var. <i>chinensis</i> , Maxim. (= <i>mandschurica</i> , W. Becker) (Miyaji in Ishikawa, 49)	*24	
<i>Patrini</i> , L. (Miyaji, 48)	36	

VI. Diffusae.

<i>diffusa</i> , Ging. (Miyaji in Ishikawa, 49)	*13	
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VII. Boreali-Americanae.

<i>cucullata</i> , Ait.	26	36-37
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Sect. DISCHIDIUM.

<i>biflora</i> , L.	6	38
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Sect. CHAMAEMELANIUM.

Erectae.

<i>glabella</i> , Nutt. (Miyaji, 48)	6	
<i>rugulosa</i> , Greene	12	39-40

Sect. MELANIUM.

I. Calcaratae.

<i>cenisia</i> , L.	10	41
<i>declinata</i> , Waldst. et Kit.	10	43-45
<i>elegantula</i> , Schott	10	42
<i>cornuta</i> , L.	11	47
<i>orthoceras</i> , Ledeb.	11	49-50
<i>calcarata</i> , L.	20	53-54
<i>Zoysii</i> , Wulf.	20	55-56
<i>Munbyana</i> , Boiss. et Reut. var. <i>Battandierii</i> (W. Becker pro sp.)	30	57

II. Tricolores.

<i>Kitaibeliana</i> , Roem. et Schult. var.	*7	58
<i>Kitaibeliana</i> , R. et S., another variety	abt.	12	59
<i>Kitaibeliana</i> , R. et S., a stout variety	18	60-62
<i>tricolor</i> , L. (Clausen, 12)	13	63
<i>alpestris</i> , (DC.) Wittr., W. Becker	13	64
<i>arvensis</i> , Murr. (Clausen, 12)	17	67
<i>rothomagensis</i> , Desf.	17	68
<i>lutea</i> , Huds.	24	69

Sect. LEPTIDIUM.

<i>Humboldtii</i> , Triana et Planch. (Heilborn, 27)	27	
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<i>Hybanthus parviflorus</i> , Baill. (Heilborn, 27)	12	
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In the *Nominium* section, Uncinatae, Rostratae, and Bilobatae belong to the 10-series. An exception is *V. canina*, with $n = 36$. Possibly, *canina* might be an octoploid species ($n = 40$), which has secondarily lost four of its chromosomes. In both Stolonosae and Adnatae, which must be regarded as closely related one to the other, a 12-series prevails. The Adnatae form a very handsome series with the values 12, 24, 36. It is interesting to note that W. Becker (8), independently of the cytological investigation, has established the new group Adnatae, which is surely a very natural one. Finally, in two other groups, we have the figures 13 and 26, which must doubtless be regarded as derivatives of 12 and 24 respectively. The two species in question, *diffusa* and *cucullata*, are probably not related in any way.

The *Dischidium* section comprises but two species. The chromosome number of the one is known; it belongs to the 6-series, as do the two species of the *Chamaemelum* section.

In the *Melanium* section the chromosome numbers are more irregular, but still group themselves about one of the cardinal numbers. I have here attempted a new form of division, taking the species investigated in two groups: Calcaratae and Tricolores. Under the Calcaratae I have included Integrifoliae, Cornutae, and Calcaratae, with the species *declinata* and *elegantula* too, the position of which has been very uncertain. Becker (3, 5) placed them in the neighbourhood of *lutea* and *rothomagensis*, but later (8) he separated them one from the other. Hayek (26) points out that *elegantula*, *declinata*, and *Orphanidis* are doubtless very closely related, but he does not seem inclined to include them in the Calcaratae group. Gams (21, p. 609), on the other hand, points out that these and others appear to occupy an intermediate position between *tricolor-lutea* and *calcarata*. And it seems to me also that it is extremely difficult to separate *V. declinata*, W. et Kit., and *V. Dubyana*, Burnat, from *V. heterophylla*, Bertol. There appear to be regular transitions from the more easterly *declinata* to the more westerly alpine *V. Dubyana*, and thence southward to types belonging to the highly variable Apennine-Greek-North African *V. heterophylla*, Bertol, which is decidedly one of the Calcaratae. On the other hand, the eastern *V. declinata* shows marked association with *V. elegantula*, Schott., *Orphanidis*, Gris., and *Nicolai*, Pant. These again seem to indicate relationship with *V. cornuta*, L., and *V. orthoceras*, Ledeb. *V. declinata* is thus, morphologically, linked up with Calcaratae, Cornutae, and Tricolores. The fact that various writers have confused *V. declinata*, *Dubyana*, and *heterophylla* one with another also strongly suggests some relationship between them. As regards *V. cenisia*, it would be most natural to include it among the Calcaratae. The entire leaves do not necessarily imply any cardinal difference from the other Pansies. The chromosome numbers themselves argue strongly in favour of the arrangement of species outlined above.

In 1926 I included *cornuta* and *orthoceras* in the 6-series, but both their appearance and the possibilities in crossing seem to suggest that *cornuta* should be referred to the 10-series in the Calcaratae group. The polymery, which is generally supposed to be in some way connected with the polyploidy, points in the same direction. In *tricolor*, which must be regarded as tetraploid, there are as a rule 2 polymeric basic genes for the violet and red colouring in flower and stem, so that a segregation would give 15 coloured : 1 *alba*. *Arvensis*, which must be regarded as hexaploid, has 3 basic genes, and segregates in the proportion of 63 coloured : 1 *alba*. *Elegantula* and *cornuta*, on the other hand, have each but 1 basic gene for violet and red, the segregation being in the ratio of 3 coloured : 1 *alba*. There is thus, as regards polymery, a difference between the *tricolor* with its 13 chromosomes and *cornuta* with 11; *cornuta* can from this be regarded as a diploid species; if 11 were a derivative of 12, it should doubtless be considered tetraploid.

The Tricolores are the most mixed of all the groups as regards their chromosome numbers. Only two or three of the eight species have a cardinal number for their chromosome number; the other figures are 6 + 1, 12 + 1, and 18 + 1. It is characteristic that 7 and 13, belonging respectively to diploid and tetraploid species, are plus-aberrations, whereas 17, which belongs to the hexaploid species, is a minus-aberration. In the hexaploid type, it is probable that all the vitally important genes are so highly doubled or trebled that the loss of a single chromosome is of no account. I have previously (15, p. 100) shown by experiment how a new constant species with 14 bivalent chromosomes can be formed by crossing a 13- with a 17-chromosomed type; similarly, it might be imagined that the 13-chromosomed type itself had originated from the crossing of a 12- and an 18-chromosome type, e.g. the 12- and 18-chromosomed *Kitaibeliana*. I have further shown how a 16-chromosomed type may arise from the loss of a chromosome after crossing two types each with 17, not altogether capable of conjugating one with another (15, pp. 47 ff.). Similarly, it might be imagined that *arvensis* originated from an 18-chromosomed *Kitaibeliana*. Possibly a *Kitaibeliana* with $n = 6$ may be the original progenitor of the Tricolores. This might be supposed to have formed, firstly, a tetraploid type with $n = 12$, and by crossing of 6×12 , on the one hand, aberrants with $n = 7$, and on the other a hexaploid type with $n = 18$ by indirect chromosome binding according to Winge's law (64, 65). And from this we can proceed farther again. It does not seem as if new dominant genes could arise, but on the other hand, crossings such as these very frequently lead to 'loss' or mutation of the dominant genes, and the recessive allelogene appears instead. The crossing analysis showed that *arvensis* contained all the dominant genes of *tricolor* and others besides; and *Kitaibeliana* will probably be found to correspond to *arvensis*—may

indeed possibly prove even more dominant than the latter. By the loss of a sufficient number of dominant inhibiting genes a *Kitaibeliana* type could doubtless be transformed into a *tricolor* as regards appearance.

The chromosome numbers afford not only an aid to the grouping of species within the different sections, but constitute also an important factor in determining whether two types are specifically distinct or not.

1. Thus *V. grypoceras*, for instance, has sometimes been noted as a variety of *silvestris*. The chromosome number, which is the same for both types, gives no ground for separating them.

2. The chromosome numbers do afford adequate reason for separating *silvestris* and *Riviniiana*.

3. *V. neglecta*, M. Bieb., was placed by Becker, in 1910, in close proximity to *V. silvestris*, as *V. Sieheana*, W. Becker; in 1923, the same writer gives it as *V. Riviniiana*, Rchb., sub-species *neglecta*, M. Bieb. Cytological investigation shows that it has the same chromosome number as *Riviniiana*, and it should therefore be regarded as at least more closely related to *Riviniiana* than to *silvestris*. From the appearance of the type, I should consider it most natural to treat it as an independent species, under the name of *V. neglecta*, M. Bieb.

4. *V. elatior* appears, morphologically speaking, to be a double *stagnina*. The cytological investigation in particular seems to suggest that it is an auto-tetraploid *stagnina* (cp. *phalacrocarpa* and *japonica* (50)).

5. The chromosome numbers provide grounds for separating *V. palustris* and *V. epipsila* one from the other as two distinct species.

6. Miyaji (48, 49) has shown that *V. Patrini*, L., has $n = 36$, whereas *Patrini*, var. *chinensis*, Maxim., has $n = 24$. Independently of this, W. Becker (6) puts forward the variety as a distinct species, *V. mandschurica*, W. Becker.

7. The close relationship indicated by outward appearance between the partners of the respective pairs: *V. elegantula-declinata*, *V. cornuta-orthoceras*, *V. calcarata-Zoysii*, and *V. tricolor-alpestris*, is confirmed by the chromosome numbers, which are alike for each of the two partners in every pair.

8. *V. arvensis* and *Kitaibeliana*, which are much alike in appearance, can be distinguished by their chromosome numbers, *arvensis* showing always $n = 17$, while *Kitaibeliana* has $n = 7$, about 12 and 18 (it is possible that still further numbers may be found in *Kitaibeliana*). *Kitaibeliana* thus consists of several types, differing in cytological respects, yet in outward appearance all typically *Kitaibeliana*. The nearest approach to a parallel here is in the case of *Draba megallanica*, Lam., in which Heilborn (28) found types with 24, 32, and 40 chromosomes. These have not yet been recognized as specifically distinct. So also Winge (66) has in three microspecies of *Erophila verna* found the chromosome numbers 7, 15, and

32, while Jørgensen (33) found in *Callitriche stagnalis* two morphologically identical types, one with $n = 5$, the other with $n = 10$, both growing wild in Denmark.

9. Gams (21) groups *V. tricolor*, *V. alpestris*, *V. arvensis*, and *V. Kitaibeliana* with all its sub-species, under one species, *V. tricolor*, just as did Linnaeus. This is surely unwarrantable also from the point of view of nature itself, which likewise separates them. The cytological investigation affords a further ground for separation, in that *arvensis* and *tricolor-alpestris* have different chromosome numbers. *Kitaibeliana* should probably be further subdivided, after careful floristic-statistic and cytological investigation.

The present investigation thus affords further proof of the great importance of cytology as an aid to taxonomic research. It forms a link in the lengthening chain of cytological investigations which have contributed to the elucidation of taxonomical questions. I would here only mention, apart from Winge's well-known work (64), the investigations of *Adoxa* (42), *Hieracium* (57), *Triticum* (59), *Erigeron* and *Eupatorium* (30), *Campanula* (46), *Lactuca* (32), *Rosa* (10, 31, 61 A), *Crepis* (2, 45, 58), *Senecio* (1), *Erophila* (65), *Betula* (29), *Rumex* (38), *Tulipa* (52), *Draba* (28), *Lamium* (34), Ranunculaceae (43), and in animals on the Drosophilidae (47).

It is indeed characteristic of modern taxonomy that it is constantly adding new methods to its means of research. Genetic investigations can also be of importance when it is desired to ascertain the distinction of species in critical genera. Kristofferson's work on *Malva* (41) and to some extent also my own *Viola* crossings (15, p. 141) afford some assistance in the distinction of species.

CHROMOSOME NUMBERS AND THE CONCEPTION OF SPECIES.

There are at the present moment two main lines discernible in taxonomy: one seeking to subdivide the species down to the smallest systematic units, the other tending in precisely the opposite direction, and reverting rather to the so-called Linnean conception of species, with its somewhat larger units. It is obvious that in our distinction of species we must first of all consider what Nature regards as units, and what not. The Mendelian principle has shown us how different types cross and exchange genes. Types differing in respect of twenty genes can, by crossing, form over a million different combinations of those genes. Once the types can be crossed fairly frequently, and segregate accordingly, it is therefore, on practical grounds, impossible to register every one of these combinations as a systematic unit. Many botanists, engaged upon the study of minor

species, will not therefore regard all the numerous combinations produced by intercrossing communities as species; they make an exception, however, in the case of apogamous clones, as these are constant. I cannot see any difference in principle between the two cases. Many of the types found in a natural state within the non-apogamous species are also constant in respect of essential characters; absolute constancy is never attained in units which propagate by alien pollination. And the facultatively apogamous types can also be crossed, thus giving rise to new apogamous types (53, 54). Given sufficient length of time, they will be capable of forming almost as many combinations as plants having sexual reproduction. I cannot agree with du Rietz (55) that there is a difference in principle between the 'unendliches Wirrwarr von Genotypen' which we find within *Viola tricolor-arvensis* on the one hand, and the enormous confusion of apogamous clones in *Hieracium*. It seems to me futile to raise all these clones to the rank of species. They are, indeed, heterozygotes of the worst kind, viz. specific hybrids. One might then just as well elevate all the numerous clones of potato to the rank of species.

But the division of species is also in full progress among the non-apogamous species. Dr. Drabble, in his works on the British Pansies (18, 19, 20), has included Jordan's old minor species and has himself added new ones. With all due respect for the pioneer work of Jordan in investigating the variation among the old groups of species, we cannot, at any rate in the case of the Pansies, take his species as the basis for our own conception of species generally. This would mean the establishment of thousands of new species at once. Owing to the facility with which *tricolor* and *arvensis*, and to some extent also other Tricolores, can cross one with another and exchange genes, a great number of types have arisen. Nevertheless, these types group themselves, as regards their chromosome numbers, into comparatively few large units, almost exclusively those with 13, 17, and 24 chromosomes, answering to the species *tricolor*, *arvensis*, and *lutea* respectively. They can also in externals be very well distinguished one from another, as is recognized in Dr. Drabble's works, inasmuch as his Arvenses comprise those types which have undoubtedly seventeen chromosomes. His groups Tricolores, Saxatiles, and Curtisieae comprise those with an undoubted thirteen. The Curtisieae answer to what is elsewhere known as *V. tricolor*, var. *maritima*; some of the Saxatiles perhaps to *V. alpestris*, (DC.) Wittr., W. Becker, though it is doubtful whether typical *V. alpestris* occurs in England. There is, however, no unfailing criterion for distinguishing *V. tricolor* from *alpestris*. *V. calaminaria*, Lej., on the other hand, which Drabble (18) places among the Saxatiles, belongs in point of chromosome number to his Luteae group ($n = 24$), and all other taxonomists are probably agreed in placing it there. The Nanae should no doubt come under the head of *Kitaibeliana*, sens. lat.

In Denmark itself we find an entirely similar multiplicity of distinctive and specialized types of *tricolor* and *arvensis*. Almost every isolated spot or island within the boundaries of the kingdom has its own type, but, save where segregation has taken place after a recent crossing of species, they fall nevertheless into two distinct groups, viz. (1) large-flowered with *labellum*, flowers violet, rose, or bright yellow, thirteen chromosomes = *V. tricolor* with its varieties; and (2) small-flowered without *labellum*, flowers yellowish white, seventeen chromosomes, = *V. arvensis* and varieties of the same. Dr. Drabble's so-called species are evidently similar local races (Drabble, 19, p. 263).

Local races occasionally reappear in several different places some distance apart. This does not necessarily imply distribution by sowing. If only the necessary genes be brought together by crossing, there is a possibility of the race being formed anew. Types resembling Jordan's races can arise in England without having had any connexion with them whatever. In 15 (p. 54) I showed how a type resembling Wittrock's *arvensis* sub-spec. *curtisepala*, var. *gotlandica*, which has *velutina* violet upper petals, arose altogether independently of the Gothland type, by the loss of a chromosome after crossing of two *arvensis* varieties from Seeland. A type answering in the main to Becker's *tricolor* sub-spec. *faerøensis*, *inter alia* in the very characteristic stipules, rather like those of *cornuta*, has been found by the present writer at Røde Kro in Sønderjylland. There is not the slightest reason to suppose that it has any phylogenetic connexion whatever with the Farøe type.

The differentiation of these local races is very easily explained:

(1) When a species finds its way to some little island or isolated spot, the very fact of its isolation restricts its opportunities of crossing. The emigrant types carry with them a certain number of genes; some of these will doubtless disappear with the types that drop out in the course of Natural Selection. And since there is but little possibility of new genes being imported from the stock in the larger area, we get, in course of time, a distinct type, which has to some extent adapted itself to its environment, as far as its original genes allow.

(2) On habitats where external conditions of an unusual character prevail, peculiar and specialized types arise, as the process of Natural Selection among the multifarious combinations formed by spontaneous crossing retains those most likely to survive in the struggle for life under the given local conditions. There may be every possibility of genes being introduced from the neighbouring areas, but the combinations in question are suppressed in competition.

The frequent establishment of species of doubtful value is very largely due to their being based principally on herbarium studies. The specimens

in the herbarium represent but a small selection from the abundance of nature. It is easy to keep a few so-called species or varieties separate as long as one is working only with herbarium specimens; but the units of classification thus established are of no use whatever in the field, as has already been pointed out by Hall and Clements (25). Nature is infinitely richer than the herbariums would seem to show. Dr. Drabble (20, p. 46) does not appear always to reckon with this abundance. C. H. Ostenfeld had, from the very extensive material in the herbarium at the Botanical Museum in Copenhagen, determined a *tricolor* type from the Orkneys as corresponding in the main to *tricolor genuina*, Wittr., var. *faerøensis* (W. Becker). Drabble, having seen a single specimen of the Faerøe type, rejects this determination. Elsewhere, however, Drabble admits (19, p. 263) that his own 'species' are also variable. Another investigator might thus with equal right subdivide Drabble's species still further. Once entered upon this road, consistency demands that one should not stop until the single combinations of variable characters are reached (Clausen (12, 13)). It is, however, out of the question, on practical grounds, to operate with so many different units, save in the case of highly specialized investigations, and even then it will doubtless be more profitable to consider the individual characters separately.

The conception of species is doubtless in certain respects determined by practical and conventional considerations, but there can be no doubt as to its having some real foundation. Nature itself does recognize certain boundaries between different sections of its manifold production, and it respects those boundaries. Varieties belonging to one and the same species form a kind of community, constantly exchanging genes by crossing; the crossing of different species, however, is of less frequent occurrence; and it is even more rarely that specific hybrids are capable of exchanging genes.

Sterility constitutes the most reliable boundary line, and this is determined as a rule by the chromosomes. Even where the sterility is only partial, there is often a difficulty in the exchange of chromosomes. Hybrids between species with different chromosome numbers are as a rule completely sterile, or if not, the distribution of the chromosomes takes place with such irregularity that there is a marked tendency to keep the original species pure (cf. 15, pp. 140-1). In any case there is nearly always impaired fertility in hybrids between species having different chromosome numbers. Sometimes, indeed, none of the chromosomes are able to conjugate. Even in species with the same chromosome number, the chromosomes of the two species may differ so greatly in their contents as to be incapable of conjugating if brought into contact. Such hybrids are, as a rule, practically speaking, completely sterile. The sterility can be removed if the hybrid doubles its chromosome number (indirect chromosome binding (64, 65)), but in this case it is a *new*, constant species

which is formed, as in the case of *Nicotiana* (16), *Aegilotrichum* (62), or *Raphanus* \times *Brassica* (35).

The chromosome number and the behaviour of the chromosomes in specific hybrids are therefore of great importance in the question as to limits of sterility. These limits should not be ignored or obscured in our delimitation of species. The old botanists worked according to their own common sense and delicate biological feeling, but they had a conception of species far more nearly coinciding with that we now arrive at by careful statistical observation of variation in the field, and by cytological investigations and crossing experiments, than our modern small-species taxonomists. 'Eine Art ist eine Art, ganz gleichgültig ob sie *Diapensia lapponica*, *Viola tricolor* oder *Hieracium marginelliceps* heisst,' says du Rietz (55, p. 241). I quite agree that it is a matter of supreme indifference to Nature what *we* decide shall be the definition of a species. But it is by no means a matter of indifference to ourselves whether we accept or reject a form of nomenclature which obscures Nature's own chief system of division. Let us name all the millions and milliards of minor variations which Nature produces, if we will, but let them be named as varieties, and not elevated to the rank of species.

W. Becker's delimitation of the *Viola* species, which is based on purely morphological considerations, appears to be sober enough, and doubtless agrees, on the whole, with Nature's own divisions; as far as the species investigated are concerned, it agrees also with the cytological features. There will always be details open to criticism in any method of division, and Becker has perhaps subdivided rather too generously; Gams, on the other hand, is inclined now and then to make do with over-large groupings. There is always room for the exercise of personal judgement in critical cases; the great thing is to restrict this indefinite field as far as possible, checking the conclusions by starting the investigations from as many points as possible.

CHROMOSOME NUMBERS AND THE PHYLOGENY OF SPECIES.

One should be very careful in entering upon phylogenetic speculations as to the possible origin of the *Viola* species. The pedigrees occasionally drawn up may safely be taken as purely arbitrary. Similarity in appearance does not necessarily imply phylogenetic relationship, for the same type can be produced by different crossings, as long as the requisite genes are brought together. Something more can be gleaned from the number and form of the chromosomes in conjunction with morphological similarities, but here also it must be borne in mind that the same result *can* be produced in different ways. I need only mention such processes as the fragmentation

of individual chromosomes (23), end-to-end adhesion of chromosomes in *Drosophila* (51), translocation of parts of chromosomes (61), species-crossing with splitting of *some* univalent chromosomes or of *all* the chromosomes, elimination of chromosomes, juxtaposition of homologous univalent chromosomes in the same zygote in species-hybrids of the *Drosera* type, and autosyndesis, where the chromosomes from one and the same parent conjugate two and two as in *Papaver* (44) and *Crepis* (17).

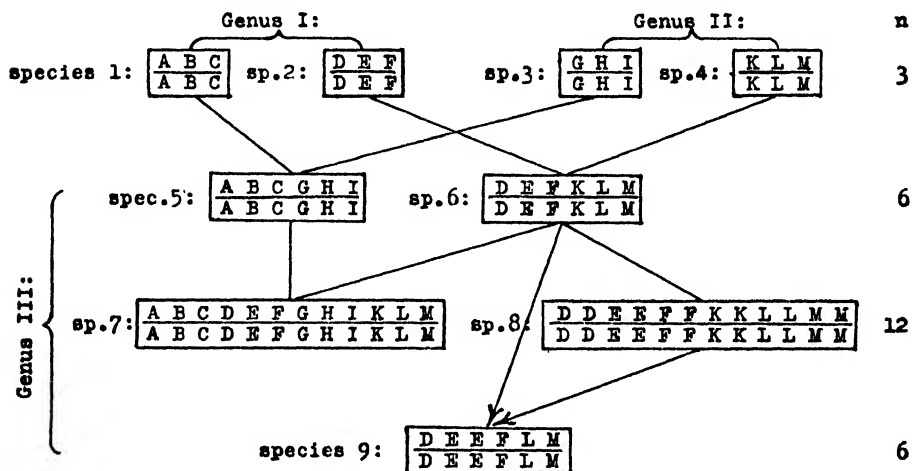


FIG. 82. Diagram representing formation on a chromosomal basis of new species and new genera. The chromosomes are indicated by capital letters, and homologous chromosomes are indicated by the same letter.

These processes are highly dissimilar in themselves, but it is quite conceivable that we may, by combined experimental genetic and cytological investigations, obtain some further insight into the phylogeny of species.

There are doubtless many processes contributing to the differentiation of a generic group into its various species. The genus itself arises perhaps through the crossing of species belonging to two other genera, as with Tschermak's tetraploid *Aegilotrichum* or Karpechenko's tetraploid *Raphanus-Brassica* hybrid. It is possible that some of the species in the new genus may actually be formed by different crossings, as shown in the diagram Fig. 82. We have here to begin with two genera, each having two species, i. e. 1 and 2 in genus I, and 3 and 4 in genus II. The species in question have each 3 bivalent chromosomes, but none of the chromosomes is homologous with any other. Crossing and subsequent indirect chromosome binding (Winge) give rise to Species 5 from 1 × 3 and Species 6 from 2 × 4. These last two may possibly have a number of characters in common, on the basis of which they may be taken together as constituting a new genus (III). Both have $n = 6$. Crossing of 5 × 6 may further produce Species 7

with 12 chromosomes. This would probably also be referred to genus III, where we have now allopolyploidy. Again, we may get an autopolyploid Species 8, with $n = 12$, formed by the doubling of the chromosomes of Species 6 without previous crossing. Winkler (67) has shown that the crossing of a homozygous *Solanum nigrum* with its experimentally produced auto-tetraploid type may give rise to a new diploid type, which is not identical with the homozygous original. This may be illustrated by Species 9, formed by the crossing of 6×8 . Species 9 is diploid, as is 6, but the *E*-chromosome is present in quadruplicate, while the *K*-chromosome has disappeared. And we cannot ignore the possibility of another process for the differentiation of species, namely, that different mutations in two isolated races of one and the same species may in course of time alter the constitution of the chromosome to such an extent that the two races no longer have homologous chromosomes. If the two races (now species) be then brought together, e.g. by alteration of climate, there may possibly arise a new tetraploid species from crossing of the two. But as mutations tend on the whole to weaken the type, it is hardly likely that it would survive so many mutations.

There can be little doubt that species-crossings in the genus *Viola* have played a great part in the differentiation of the genus. The species of the *Melanium* section are doubtless relatively young, Natural Selection not having yet exterminated so many combinations as to leave the remainder separated by boundaries of absolute sterility.

Characteristic of the *Melanium* section also are the numerous slight deviations in the chromosome numbers from the cardinal figures. At the same time, it is remarkable that the chromosome numbers of the different species group themselves in the vicinity of the cardinal numbers. This is the more noteworthy when we consider the frequent species-crossings and the manifold processes which they can lead to, as mentioned above (p. 708). I have dealt with this question in detail in my paper on *arvensis* \times *tricolor* (15).

This is presumably connected with the fact that the chromosomes in the pure species form a harmonic system. Every species is subject to the action of a number—doubtless a very large number—of genes, acting to some extent one against the other. The course of the physiological processes, like the morphological characters, is doubtless partly determined by the co-operation of a series of genes, both those with inhibiting effect on the processes themselves, and those which accelerate the same. In the pure species all these manifold effects of contrary processes are balanced to a nicety, or the species would never have survived in the struggle for existence. The genes responsible for these effects are located in the chromosomes. If all the chromosomes be duplicated at once, then all the genes are likewise duplicated, and the balance is not disturbed thereby. But when

we have a species-crossing with subsequent interchange of chromosomes between the two species, producing new combinations of the chromosomes and genes, then it is not certain that all these combinations will be balanced; possibly only the combination of the parent types and a few others will be in equilibrium, and thus fitted to survive. Should there be a number of univalent chromosomes in the species-hybrid, then it may be that the types are only fully balanced as long as they have all or none of the univalent chromosomes. For, as the polyploid series are as a rule doubtless formed by the duplication of *whole sets* of balanced chromosome combinations, it follows that, as a rule, only those types which contain either whole or approximately whole sets will be so balanced. Kihara (37) has shown this very neatly in the hybrid between a 14-chromosomed and a 21-chromosomed *Triticum*, and Karpechenko (35) has shown, in the case of *Raphanus-Brassica* hybrids, that it was actually only those *gametes* which contained entire or nearly entire sets of the chromosomes from the two parent types which were able to survive. In the case of *Viola*, Nature is not so particular; the chromosome numbers found, however, seem to show that the species do not allow of any marked deviation from the cardinal numbers.

It would be very desirable to have knowledge of the chromosome numbers of the majority of species in a large genus having representatives throughout the globe. The genus *Viola* is suitable in this respect, and forms an interesting object for cytological study; up to the present, however, only 40 of its species—some 500 in all—have been investigated. It is difficult to procure seeds of the pure, wild-growing species. The best way would be for several cytologists in different parts of the world to investigate each those species which he was best able to collect and observe. I should be interested to know whether any others would care to take up such investigations. In the course of the next few years I purpose myself to continue the work I have already begun, a comparative genetic-cytological investigation of a series of species-hybrids in the *Melanium* section; but I should always be glad to receive viable seeds collected from wild species of *Viola*, for determination of the chromosome numbers. I wish to express my hearty thanks to Dr. G. Voronov, of the Tiflis Botanical Gardens, and to Professor A. Ernst, who have kindly furnished me with seeds of some of the most interesting *Viola* species.

SUMMARY.

1. The present work deals with an investigation of the chromosome number of some 30 species of *Viola*. The numbers for these species, as well as for those investigated by other writers, are given in the table, pp. 698–9.
2. At the moment, the following chromosome numbers are known for *Viola* species: 6, 7, 10, 11, 12, 13, 17, 18, 20, 24, 26, 27, 30, and 36. They

fall, however, more or less into definite groups, forming a 6-series, a 12-series, and a 10-series. In the *Nominium* section there are a 10- and a 12-series; in the *Melanium* section a 10- and a 6-series, the latter, however, with rather numerous aberrations.

3. In *Viola Kitaibeliana* three chromosome numbers have been ascertained up to the present, viz. 7, about 12, and 18, all for Caucasian types (p. 691).

4. Species of the same systematic sub-group belong as a rule to the same series of chromosome numbers (pp. 698-702).

5. In the *Melanium* section, a new subdivision has been made on the basis of investigation of chromosome numbers, the section being now divided into two groups: the *Calcaratae* (the 10-series) and the *Tricolores* (the 6-series).

6. *Viola elegantula* and *declinata* are transferred from the *Tricolores* to the *Calcaratae*, as both chromosome numbers and morphological characters show that they most naturally belong there (p. 700).

7. On pp. 702-3, under headings 1 to 9, I have enumerated those cases in the genus *Viola* where the chromosome numbers found afford indications as to whether the systematic units in question are different species or not.

8. The cytological conditions of some spontaneously occurring species-hybrids of *Viola* are described, viz. *Riviniana* × *silvestris* (p. 699), *hirta* × *odorata* (p. 684), *epipsila* × *palustris* (p. 684), and *arvensis* × *tricolor* (p. 692). Furthermore, the following artificially produced species-hybrids are mentioned: *Violacornuta* × *elegantula* (p. 688), *alpestris* × *tricolor* (p. 692), and *lutea* × *tricolor* (p. 694). In *epipsila* × *palustris*, *lutea* × *tricolor*, and probably also *Riviniana* × *silvestris*, the univalent chromosomes split in the heterotypic metaphase.

9. The cultivated Pansies produced by species-crossing between *Viola lutea* and *tricolor*, and between these and *cornuta*, have as a rule $n = 24$ (approx.), but the gametes in one and the same plant may have different chromosome numbers (p. 696). Further, mention is made of some few other cytologically irregular types from botanical and other gardens (pp. 688 and 696).

10. Several questions of theoretical interest are dealt with in detail: the behaviour of chromosomes in specific hybrids (pp. 697-8), the chromosome numbers and classification of species (pp. 698-703), the conception of species in relation to the number of chromosomes (pp. 703-7), and lastly the chromosome numbers and phylogeny of the species (pp. 707-10).

For some years past, the Carlsberg Foundation has granted me financial support in aid of these *Viola* investigations, of which those now published constitute a part. I beg to express my very sincere thanks to the governing body of the Foundation for this help. Most of the

species investigated were cultivated in the University Botanical Gardens, Copenhagen. I am very grateful to the authorities and staff for the generous help and space accorded me for the work. The text of the present paper is translated from the Danish original by Mr. W. Worster, M.A.

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Peculiarities in the Structure of the Stem, related to the Leaf-sheath, in *Hedyosmum*.¹

BY

ALEXANDER F. SKUTCH.

With Plate XXX and twenty-four Figures in the Text.

INTRODUCTION.

HEDYOSMUM ARBORESCENS, Sw., a representative of the single American genus of the Chloranthaceae, occurs throughout the West Indies and in Brazil (5), and is fairly abundant in the montane rain forest of the Blue Mountains of Jamaica, where specimens were collected by the writer during the summer of 1926. Here the plant is usually a small tree, with extremely hard wood. The largest tree measured by the writer had a girth of 90 cm. at a height of 30 cm. from the ground, but most of the examples observed were very considerably smaller than this. The rather large, thick, and glossy, oblong leaves and the smooth and shining green covering of the younger twigs give to these plants a very pleasing aspect, even in the dark glades where they are native.

The short petioles of each pair of the opposite and decussate leaves spring from near the upper margin of a rather massive and succulent tubular sheath, formed by the connate bases of the leaves (Pl. XXX, Fig. 1). In mature branches, the sheath completely surrounds the stem for a distance of 14–19 mm. above its insertion on the latter. In the case of strong shoots, the lateral branches become free of the axis at some distance above the upper margin of the sheath. This means, of course, that a considerable length of stem must intervene between the axil of the leaf and the point of departure of the lateral branch, a length which not infrequently amounts to 27 mm. Following the convenient terminology of Schumann (11) we shall refer to this separation of the branch from its subtending leaf as *extra-axillation*.

The altogether unusual degree of extra-axillation in *Hedyosmum* seems generally to have escaped the notice of botanical writers. Clarke (2) refers briefly to the resemblance of the jointed stems of the Chloranthaceae to

¹ Botanical contribution from the Johns Hopkins University, No. 89.

those of the Polygonaceae, but does not mention any peculiarities of branching. Engler (4) briefly states that in the Chloranthaceae the opposite branches are fused with the stem, but this circumstance is omitted entirely from some other systematic works. It is of interest to compare the degree of extra-axillation of *Hedyosmum* with that of other plants. The writer has observed that in strong terminal shoots of saplings of the hickory (*Carya alba*, (L.) K. Koch) the uppermost and strongest of the three buds which often occur in longitudinal series above the axil of the leaf is at times separated from the latter by 8, 9, or more rarely 10 mm. In the honey locust (*Gleditschia triacanthos*, L.) the branched spine which represents the most distal of the superposed multiple buds is sometimes removed from the axil by 10 mm. According to Russell (10), in *Vitex agnus-castus* the uppermost of the multiple buds becomes free from the stem at almost 1 cm. above the axil of its subtending leaf. In *Celastrus lucidus* the axillary bud develops into a spine, while an accessory, which Russell considers to be a precocious branch of the former, grows out into a leafy shoot, and is at times displaced upward on the main axis to 1 cm. above the spine. In none of these cases, however, does the degree of extra-axillation approach that to be observed in *Hedyosmum*. In that classical and much-discussed example of the separation of the branch from its subtending leaf, the floral peduncle of *Anchusa* and *Symphytum* in the Boraginaceae, the degree of extra-axillation exceeds that in our plant. In *Anchusa italica*, according to Schumann (11), the peduncle may spring from the axis in the third internode above the leaf in the axil of which, as he demonstrated by dissection of the growing-point, the primordium which gives rise to it is laid down (see also Goebel (6, Fig. 1393)).

In most cases in which there occurs a considerable separation between bud and subtending leaf, this is associated with the production of multiple buds, and often, as in *Gleditschia* and *Celastrus*, with the morphological specialization of one of these buds, as into a spine (see also Cook, (3)). In *Hedyosmum*, on the contrary, the extra-axillation seems to be primarily a result of the readjustment of organs made necessary by the presence of the massive and persistent foliar sheath. The inferior accessory bud present in *Hedyosmum* usually remains small and dormant; it occupies but an inconsiderable proportion of the space between the primary bud and the axil of the leaf, and is itself often removed from the latter by more than 1.5 cm. This sheath, therefore, deserves a more detailed consideration.

THE SHEATH.

Each branch is terminated by the cylindrical foliar sheath, which pushes upwards beyond the growing-point and encloses it. The sheath which prolongs the shoot is not readily distinguishable from the latter by external appearance so that one receives the impression that the shoot is

10-15 mm. longer than it really is, for the growing-point may be quite this distance behind the apparent insertion on the stem of the terminal pair of leaves, which is actually their insertion on the end of the sheath. The development of all portions of the leaves, including the sheath, proceeds far more rapidly than the elongation of the growing-point, so that when the leaves which appear to terminate the shoot are almost mature, the growing-point does not extend much above the base of the long sheath, and the next younger pair of leaves is hidden inside and near the base of this structure (Text-fig. 1).

The sheath at this stage is, especially in its more distal portion, practically a solid cylinder. A careful examination of the space between the petioles of the terminal pair of leaves reveals, not a stem growing-point, as might on first sight be expected, but a narrow slit, transverse to the line joining the two petioles, across the upper end of the sheath. At each end of this slit are two tiny awns, to which we shall refer again later. A cross-section of the upper portion of the sheath discloses a roughly I-shaped opening in the centre of the broadly elliptical tissue mass (Text-fig. 5). It is through this passage-way that the succeeding pair of leaves must push upward in order to expand. The pressure which these younger leaves exert from within widens the slit into an elliptical opening just wide enough to accommodate them (Text-fig. 4). The mechanical significance of the folds of the inner epidermis of the sheath, forming the cross-arms at the ends of the long slit in Text-fig. 5, is at once evident. The central passage of the sheath is destined to widen greatly, at the same time increasing the area of its walls. By the straightening out of these folds a portion of the increase in the size of the passage is accounted for, and the amount of growth which must be made by tissue already nearly mature is correspondingly diminished.

The terminal sheath affords the growing-point and the younger leaves their only protection against injury from the exterior, since there are no bud scales or other protective structures of any kind. The slight lips at its upper extremity tightly close the narrow passage in the centre of the sheath. The characteristic veneration of the paired leaves as they push upward through the sheath is reproduced in Text-fig. 4. The terminal leaves of weak shoots are sometimes reduced to the sheath alone, surmounted by two small lappets which represent the petioles and blades, a condition which is reminiscent of the reduction of leaves to bud scales in our temperate woody plants.

As to the morphological nature of the sheath, the writer regards it as the connate and elongated leaf bases of the opposite leaves. The fusion of the leaf bases into a ring of tissue encircling the stem is so common in opposite-leaved dicotyledons that examples will occur to every one. If one imagines, for example in *Lonicera sempervirens*, L., the elongation, to several score times their present height, of the connate leaf bases in contact with

the stem, one will have a condition closely resembling that in *Hedyosmum*. The sheath has no proper bundles, and has no vascular supply other than the seven or more bundles which traverse it on either side on their way to the petiole (see Text-figs. 2, 4, 5, 9, 10, and 11). In ontogeny, the development of the sheath follows that of the laminae, which are already completely formed and are fairly large when the former becomes visible as a low rim surrounding the growing-point (Text-figs. 1 and 16). The slight rim above the insertion of the petioles (Text-fig. 2) is secondary and results from the mechanical pressure of the expanding stem upon the upper margins of the slit, forcing them upward; it is not evident in the terminal sheath of a shoot. Two slight teeth on either side of the sheath (Text-fig. 15) are regarded as stipular; they closely correspond in position to the stipular teeth of some of the Rubiaceae, e.g. *Psychotria*. The sheath in *Hedyosmum* is accordingly not in any way homologous to the ochrea or stipular sheath in the Polygonaceae.

THE LATERAL BUDS AND BRANCHES.

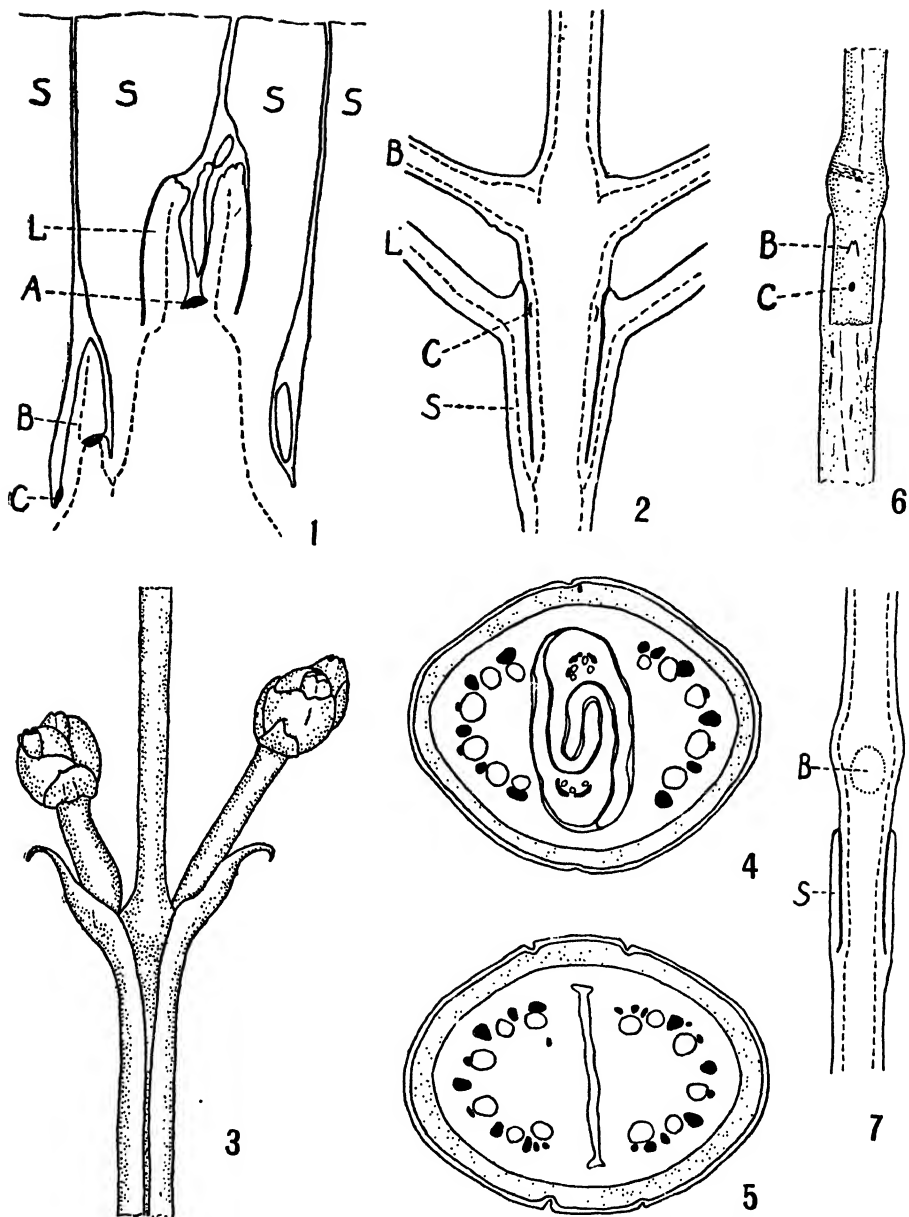
When first visible to the naked eye, the lateral buds are at the axils of the leaves, as in most other dicotyledons. At successively older nodes the process of extra-axillation may be followed, until at the fourth or fifth node

TABLE I.

Measurements of a Strong Shoot.

Col. 1. No. of node and inter- node above	Col. 2. Length of internode.	Col. 3. Length of sheath.	Col. 4. Distance from axil to free por- tion of the lateral buds or branches.	Col. 5. Distance from top of sheath to free por- tion of the lateral buds or branches.	Col. 6. Distance from axil to acces- sory buds.	Col. 7. Length of la- teral buds or branches.
	Terminal bud =					
	5.5 mm.	17 mm.	—	—	—	—
2	6 "	17 "	0 mm.	— 7 mm.	Not visible	8 and 8.5 mm.
3	65 "	19 "	14 "	— 5 "	10 mm.	51 and 59 "
4	72 "	19 "	19 "	+ 0 "	11 & 12 "	53 and 56 "
5	122 "	17 "	21 "	+ "	18 "	162 and 213 "
6	116 "	18 "	20 "	4 "	16 "	162 and 274 "
7	97 "	18 "	21 "	3 "	13 "	—
8	110 "	Fallen	18 "	—	15 "	—

behind the growing-point the lateral buds reach practically their maximum separation from the axils. Table I, column 4, gives the distance from the point of departure of the successive branches to the axil of the subtending leaves. In column 3 are recorded the lengths of the sheaths of these leaves, and in column 5 are indicated the distances from the apparent insertion of the buds, or the branches developed from them, to the upper



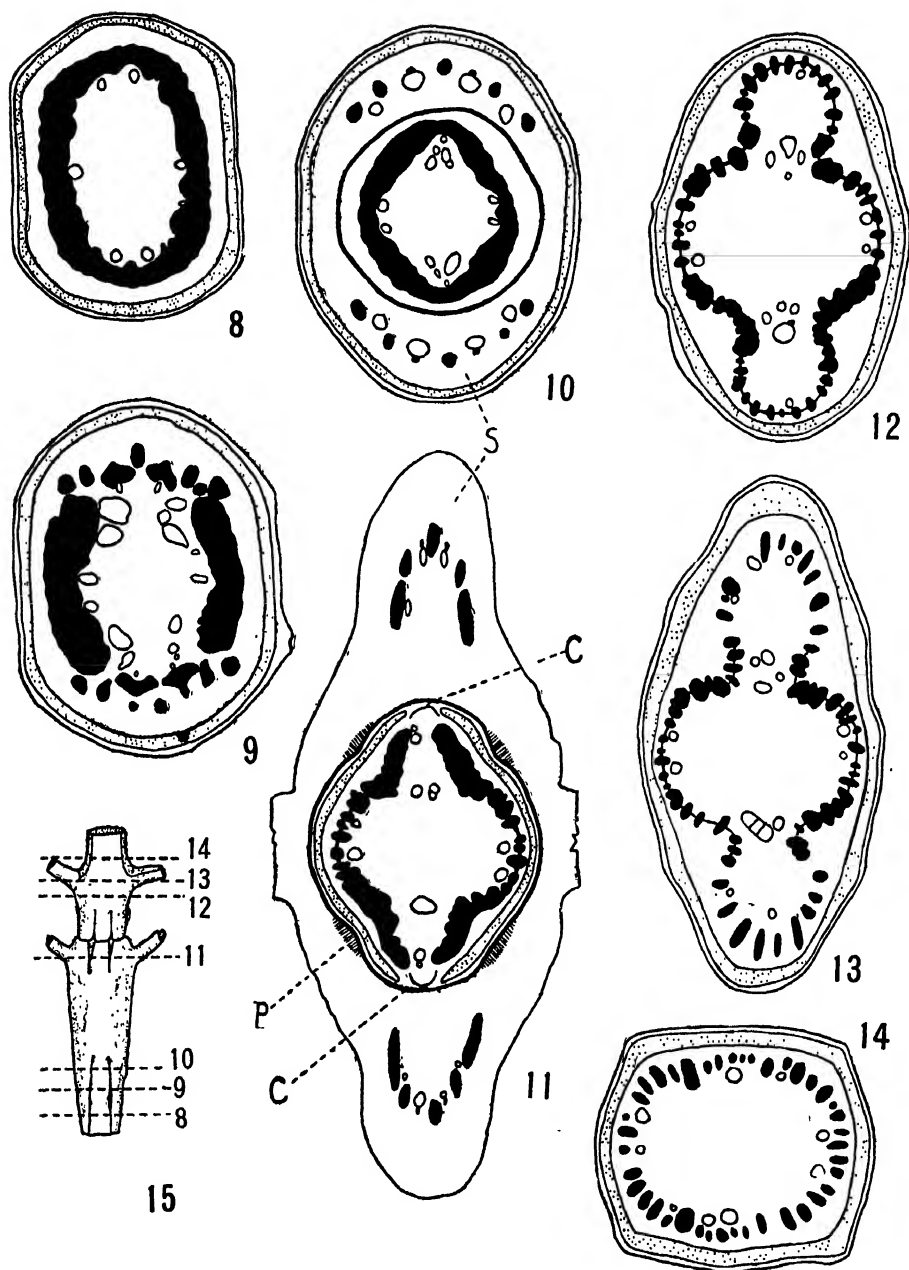
TEXT-FIGS. 1-7. Fig. 1. Longitudinal section through the growing-point of a vigorous shoot ($\times 14$). Fig. 2. Longitudinal section through a portion of the shoot which has ceased to elongate, but has not yet experienced much secondary growth in thickness; diagrammatic ($\times 1.5$). Fig. 3. Sketch of a portion of the infrutescence, showing the decurrent bracts ($\times 3$). Fig. 4. Cross-section of the basal portion of a terminal sheath through which the next younger pair of leaves is pushing upward ($\times 9$). Fig. 5. The same sheath higher up, above the tip of the young leaves ($\times 9$). Fig. 6. A weak branch, of which the lateral bud is well covered by the sheath. The sheath has been cut away on the side towards the observer. The swelling just above the sheath is the pulvinus ($\times 1$). Fig. 7. Portion of a stem similar to that in Fig. 2, but cut in a plane normal to the branches, to show the pulvinus ($\times 1$). A = apical growing-point; B = lateral bud or branch; C = accessory bud; L = leaf; s = sheath. In Figs. 1, 2, and 7 the course of vascular bundles is indicated by broken lines, primary meristems by solid black. In Figs. 4 and 5 collenchyma is stippled, mucilage passages are indicated by open circles, vascular bundles by solid black. Figs. 1, 4, and 5 are camera lucida sketches.

margin of the sheath. In column 7 are remarks on the degree of development of these lateral buds. It may be well to add that the figures recorded in column 4 do not indicate the maximum degree of extra-axillation (27 mm.) which has been observed.

This separation of the point at which the branch becomes free of the stem from the axil in which it arises may conceivably be accomplished by any one of three processes. (1) There may be a direct fusion between the main branch and its originally free lateral. Such a fusion should be immediately revealed by peculiarities in the arrangement and orientation of the vascular bundles in the region of fusion. (2) The inception and activity of an intercalary growing-zone *between* the insertion of the bud and that of the leaf may produce the separation of the two. Such a zone might arise, for example, along the line γ - γ in Text-fig. 16. According to Kolkwitz (8), the extra-axillation of the peduncle in *Anchusa* and *Symphytum* is accomplished in such a manner. (3) There may occur an elongation of the region of insertion of the lateral bud on its relative primary axis. This, in the object of our inquiry, would mean the elongation of the length of stem between the lines x - x and γ - γ in Text-fig. 16 (or a part of this length). A similar process is known to occur widely among the higher plants, and has been called upon by Čelakovský (1) to explain the extra-axillation of the peduncle in *Anchusa*. Goebel (6) holds this to be the general mode of production of leaf-borne buds and of bracts raised upon their axillary shoots, and Russell (10) considers this to be the mode of extra-axillation in the case of multiple buds.

Text-fig. 1 represents a longitudinal section through the growing-point of a shoot. The section shows the bud B, which is axillary to the sheath at the third node below the growing-point A, counting L and its companion as leaves arising from the first node. Unfortunately, the section did not pass directly through the companion bud across the stem from B, but it did slice off a portion of one of the leaves belonging to this bud. The bud B is inserted wholly on the axis, corresponding to scheme I, p. 121, in the Bonn Text-book (12). At the base of the bud B may be seen the rudiment of the accessory bud C, which is as yet a portion of the undifferentiated lens-like mass of meristematic tissue indicated in black. Text-fig. 2 represents a longitudinal section through a portion of the stem in which growth in length has ceased; the branch B corresponds to the bud B in the preceding figure. The accessory bud C now occupies a position near the top of the sheath. This bud will serve as a landmark in tracing the development of the stem between x - x and γ - γ (Text-fig. 16).

Text-figs. 8-14 represent a series of transverse sections from just below the insertion of the sheath to just above the departure of the branches of a portion of the stem in which elongation has ceased. A certain amount of secondary thickening has occurred, especially in the lower portion of this



TEXT-FIGS. 8-15. Figs. 8-14. A series of cross-sections ($\times 6$) through the stem shown in Fig. 15 ($\times 1$) at the indicated levels. C = accessory bud; p = periderm; s = sheath. Collenchyma is stippled, mucilage passages are indicated by open circles, vascular bundles by solid black, periderm hatched. In Fig. 15 the sheath extends from just above the line 9 to above the line 11. All figures except 15 from camera lucida sketches.

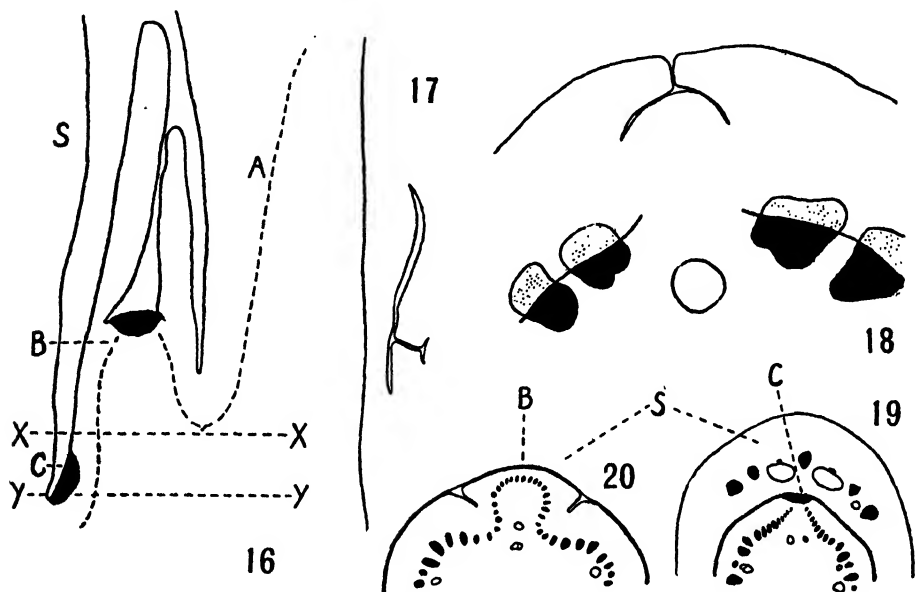
length of stem, and this makes it more difficult to trace the primary bundles ; but the essential relations remain unchanged from the primary condition. Text-fig. 8 shows the arrangement of the bundles at the level 8 in Text-fig. 15, just below the insertion of the sheath. Text-fig. 9 is through the insertion of the sheath, and shows the departure of the five large and two small bundles which supply the leaf. The leaf-gap is partially closed by secondary vascular tissue. In Text-fig. 10 the circle of vascular tissue is again completely closed. The section in Text-fig. 11 passes through the accessory buds C, the circle of vascular bundles begins to elongate towards the sides of the stem on which the branches are inserted. In Text-figs. 12 and 13 the departure of the numerous branch traces may be followed. In Text-fig. 14, above the insertion of the branches, we have a repetition of the same picture which 8 would have presented before the initiation of the cambium. The very gradual bending outward of the branch traces is also shown diagrammatically in Text-fig. 2.

This series of sections shows no abnormal arrangement of bundles. The orientation of all the bundles is strictly normal, and accordingly it has not been considered necessary to indicate the xylem and phloem separately in the text-figures, which are drawn with very slight enlargement. The first of the three alternative schemes suggested above therefore derives no support from anatomy, nor from developmental studies, and it must be rejected.

In the series of sections chosen for reproduction, the departure of the branch traces occurs gradually through over 2 cm. of stem length. Had the sections been cut through a young lateral bud, such as that shown in Text-fig. 16, an exactly comparable series could have been secured from about 1 mm. of stem length, and sections corresponding to Text-figs. 10–13, but without any secondary thickening of the bundles, could have been secured from the region between X–X and Y–Y in Text-fig. 16. Text-fig. 19, cut from a similar bud, corresponds to Text-fig. 11 from the fully elongated stem ; Text-fig. 20 corresponds to Text-fig. 12. It does not seem necessary to present more stages for comparison. The great similarity between the two series of sections, the one from the elongated stem, the other through the insertion of the lateral bud while it is still axillary, as well as *the removal of the accessory bud to a position about midway between the axil of the leaf and the free portion of the lateral branches*, seem to be conclusive evidence of the nature of the length of stem between the insertion of the leaves and the departure of the branches. It represents the elongation of the region of the insertion of the bud to its relative main axis, and agrees with the third of the alternatives given above. Such a condition is sometimes loosely spoken of as ‘fusion of the branch with its axis’. In a stricter sense, there has been no fusion, as between originally distinct organs or portions of organs. What has occurred is the elongation of a region where the two organs were already

in contact (the region between X-X and Y-Y in Text-fig. 16). This condition may be more accurately designated as 'the elongation of the region of fusion'.

It is evident that in *Hedyosmum* a very considerable portion of the increase in length of the axis is accounted for by the intercalary growth of



TEXT-FIGS. 16-20. Fig. 16. Longitudinal section through the lateral bud shown in Fig. 1, but more enlarged ($\times 39$). Fig. 17. Longitudinal section through an accessory bud of the age shown in Fig. 2 ($\times 39$). Fig. 18. Cross-section through an accessory bud of about the same age ($\times 39$). Fig. 19. Cross-section through an accessory bud of about the age shown in Fig. 16, C, and about the level of Y-Y ($\times 9$). Fig. 20. A cross-section of the same stem at a higher level, corresponding approximately to the line X-X in Fig. 16 ($\times 9$). Same lettering as in Figs. 1-7. In Fig. 18 the xylem is solid black, the phloem stippled. All figures from camera lucida sketches.

a very restricted region of a portion of the node. This may be compared with the special growing-region above the node in grasses, or that just below the node in certain mints. However, in *Hedyosmum*, when the region in question has reached, say, a third or a half of its final length, no special portion of it is set aside as an intercalary meristem, but growth is diffused throughout its entire length.

Up to this point we have confined our attention to strong shoots of which the lateral buds are carried upward considerably above the sheath, and develop into vigorous branches. With weak and suppressed shoots the situation is greatly different. Here the lateral bud, which remains small and is closely appressed to the axis, does not immediately develop, and is seldom carried upward as far as the top of the sheath. Often it may remain only 2 or 3 mm. above the axil (see Table II and Text-fig. 6). Such a dormant bud may develop later, when the sheath which surrounds it

has fallen away. Of even greater interest are cases in which one bud develops and its companion on the other side of the stem remains dormant. Here the developed bud or branch extends considerably higher up the stem than its suppressed neighbour. Thus in one example the branch became free of the axis at 15 mm. above the axil, while the bud across the stem reached only 8 mm. above the axil (see also Table II, column 4).

TABLE II.

Measurements of a Weak Branch.

Col. 1. No. of node and inter- node above it.	Col. 2. Length of internode.	Col. 3. Length of sheath.	Col. 4. Distance from axil to free por- tion of the lateral buds.	Col. 5. Distance from top of sheath to free por- tion of the lateral buds.	Col. 6. Distance from axil to acces- sory buds.	Col. 7. Length of la- teral buds or branches.
	Terminal bud =					
1	18 mm.	16 mm.	—	—	—	—
2	12 "	17 "	0 and 1 mm.	-17 and -16 mm.	Not visible	1 mm.
3	58 "	15 "	15 "	0 "	9 and 8 mm.	38 and 54 "
4	21 "	15 "	3.5 "	-11.5 "	1.5 "	2 "
5	24 "	14 "	4 "	-10 "	Not visible	1.5 "
6	30 "	Fallen	4 and 5 "		" "	1.5 "
7	25 "	"	4 "	—	" "	1 "
8	16 "	"	2 "	—	" "	1 "
9	31 "	"	4 "	—	2 mm.	1 "
10	32 "	"	3 "	—	1 only, 1 "	1 "
11	37 "	"	2.5 "	—	1 "	1 "

Whether it is necessary, following Kolkwitz (8, 9) in his explanation of extra-axillation in *Symphytum*, to assume the presence of curved growing-zones, or whether there is a difference in the time of initiation of the two buds, was not determined. It is not uncommon, in opposite-leaved plants, to find that the leaves and buds at certain nodes are not exactly opposite.

The inflorescence in *Hedysmum* forms a loose panicle. The leaves subtending the basal branches are differentiated into petiole and lamina, and form a distinct but reduced sheath. The branches are extra-axillary by about 4 or 5 mm. In the case of the more apical portions of the inflorescence the branches arise directly from the axils of the lanceolate bracts. These bracts are decurrent on the axis for some distance into the internode below. Such decurrent leaf bases are held by some writers to indicate a fusion between the leaf and the stem, or, in other words, the cortication of the axis by foliar tissue (1, and the literature there cited). Accepting this point of view, we are able to bring the condition observed in the inflorescence into closer relation with that described for the vegetative shoot. In the latter, a length of stem below each pair of lateral branches

is surrounded by a foliar tissue in the form of the sheath, which, however, is free from the stem. In the inflorescence, the axis below each pair of branches is also surrounded by foliar tissue, which in this case is fused with the tissue of the stem (see Text-fig. 3).

THE ACCESSORY BUD.

Between each main lateral bud and the axil of the leaf there normally occurs a single accessory bud (Text-fig. 2, C). The first recognizable rudiment of this bud is a lens-like mass of meristematic tissue situated in the axil (Text-figs. 1, 16, and 19, C), but subsequent intercalary growth results in its upward displacement almost to the top of the sheath. Usually the accessory buds do not develop, but remain dormant as an organ reserve, and so long as they are surrounded by the sheath they do not protrude beyond the general level of the surface of the stem. Superficially, they are recognizable only as small brownish areas in the surface of the stem (Text-fig. 6, C). The growing-point is sunken beneath the surface; from median longitudinal sections, such as Text-fig. 17, one might also take this bud to be endogenous, but cross-sections (Text-fig. 18) reveal the scale-like rudimentary leaves which have grown around and buried it. This sunken position of the bud is a result of the pressure exerted by the sheath upon the organs within it.

In the event of injury to or loss of the branch just above it, the accessory bud may become active and grow out into a replacement shoot (Pl. XXX, Fig. 2).

Russell (10), from his extended study of multiple buds, including most of the familiar examples of this phenomenon, concluded that in all cases observed by him the supernumerary buds are precocious branches of the principal or axillary bud, and propounded the law of the unity of the axillary bud. We have seen that in *Hedyosmum* the accessory bud arises in most intimate connexion with the principal bud, before the latter has formed rudiments of other branches. The writer is therefore inclined to regard the accessory C as a precocious branch of the principal bud B, and consequently a lateral of the second order in respect to the axis A. In the present instance this explanation does not seem to him to be at variance with the view of Goebel (6, p. 1455), that the accessory bud arises from an intercalary meristem remaining between the principal bud and its subtending leaf.

RELATION BETWEEN THE SHEATH AND THE STEM.

The mechanical relation between the sheath and the structures which it surrounds is at all stages most intimate. As noticed above, the central passage of the sheath is tightly closed until the next younger pair of leaves force it apart to create a passage for themselves. Later, the stem

follows the leaves upward, and the passage is further dilated. The lateral buds fit into niches hollowed out in the sides of the stem, and do not project beyond its general level. As may be seen from Table I, the lateral buds of vigorous shoots grow out into branches before they have been raised above the top of the sheath. In this event, also, the three axial members are closely crowded within the cylindrical cavity of the sheath, which is sufficiently massive to resist distortion by them. As a result, the main axis becomes biconcave in cross-section, while the cross-section of each of the two lateral branches resembles a biconvex lens. These three figures fit together to form roughly a circle, which closely fits the outline of the sheath.

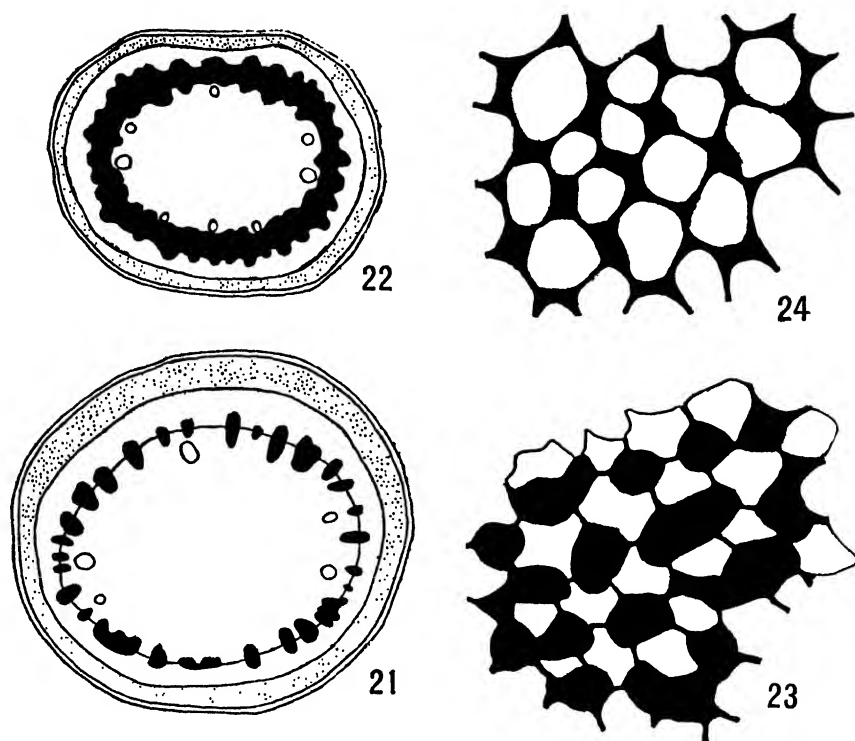
The sheath is provided throughout most of its length with a well-developed collenchyma, which abuts on the outer subepidermal layer. It is therefore well fitted to support the tender tissues of the stem within it, which, while still in the stage of active growth and with the mechanical tissues still undeveloped, must in many cases bear two or three mature leaves which are distal to it. In order to be effective in supporting the stem, it is essential that the sheath remain in close contact with it. The marked contraction of the stem where it enters the sheath from above is evidence of the closeness of this union (Text-fig. 2). In fully elongated nodes, a furrow develops on either side of the insertion of each lateral branch (Text-fig. 11). Near the top of the sheath, each furrow is filled in by a pad of phellem derived from the activity of a phellogen of subepidermal origin, so that the contact between the stem and the sheath is maintained despite the local concavity of the former. This periderm is quite local, and at the level of Text-fig. 11 was confined to the regions indicated by the hatching. A complete ring of periderm may also arise on the inner side of the sheath near its base, and in younger sheaths a great deal of phellem is often formed locally to fill in a space left empty by the developing organs of the terminal bud. Lenticels are formed on the exterior surface of the sheath through the activity of a subepidermal phellogen.

THE PULVINUS.

On weak branches, of which the dormant lateral buds have not pushed above the sheath, a very pronounced swelling of the stem is localized just above the free margin of the sheath. Thus in one stem the diameter at the swelling was 7.5 mm., while just above and just below the diameter was only 5 mm. (Text-fig. 6). The external appearance of this nodosity strongly suggests that we have here a pulvinus specialized to perform movements of curvature which result in placing the stem in better orientation to its environment. This supposition is supported by the fact that one often sees stems more or less strongly flexed at the pulvinus but straight in the intervening lengths.

The pulvinus differs in its anatomy from the adjacent regions of the stem in the following features:

1. The great dilation of the central pith at this point (compare Text-fig. 21 with Text-fig. 22).
2. The delayed production of secondary vascular tissues. In the pulvinus of *Hedyosmum* the vascular bundles are much more widely separated



TEXT-FIGS. 21-4. Fig. 21. Cross-section through the pulvinus of a stem with undeveloped lateral buds ($\times 9$). Fig. 22. The same stem just above the pulvinus ($\times 9$). Fig. 23. A few collenchyma cells from the pulvinus ($\times 165$). Fig. 24. Collenchyma cells from a neighbouring region of the stem ($\times 165$). In Figs. 21 and 22 the collenchyma is stippled, the vascular bundles are solid black, the mucilage passages are indicated by open circles. All figures from camera lucida sketches.

than in neighbouring regions. The initiation of the cambium, and accordingly the production of secondary xylem and phloem, lags behind that in adjacent regions. At the pulvinus illustrated in Text-fig. 21, the interfascicular cambium is just beginning to become differentiated from the procambial cells. Just above the pulvinus, and therefore in a slightly younger portion of the stem (Text-fig. 22), the cambium has produced considerable secondary vascular tissue and formed a complete ring of wood and phloem.

3. The absence of mechanical tissues. A strand of very hard bast fibres, in contact with the outer surface of the phloem, accompanies each

vascular bundle. At the pulvinus the cells which elsewhere form these fibres remain thin-walled, and the mechanical elements are accordingly absent.

4. The relatively great development of the collenchyma. A comparison of Text-figs. 21 and 22 illustrates the greater thickness of the collenchyma ring at the pulvinus. Micrometer measurements show that the collenchyma is approximately twice as thick in the pulvinus as elsewhere in the stem. However, it is not only in the number of collenchyma cells forming the ring that the collenchyma here differs from that of the rest of the stem; the thickening of the walls of the individual cells is also much more pronounced here (compare Text-figs. 23 and 24).

In strong shoots where the lateral branches are well developed a pulvinus presenting the same anatomical features occurs at, or slightly above (Text-fig. 7), the level of the apparent insertion of these branches. Because of the dilation of the stem associated with the departure of the branches, the pulvinar swelling is not so noticeable here as it is in the case of shoots with suppressed lateral buds, and for this reason the latter case has been treated first. However, a more careful scrutiny reveals that the pulvinus is equally well developed here, and it stands out clearly in stems halved longitudinally in the plane normal to the two branches (Text-fig. 7). Each branch is also provided with a basal pulvinus (see Text-fig. 2).

The occurrence of the pulvinus on the stem at a point considerably separated from the foliar node is a condition which is very rare in other plants. Goebel (7, p. 62) records the development of the pulvinus near the middle of the internode in *Pilea stipulosa*, Miqu., and remarks that such a situation is confined to a very few species of plants. The anatomical peculiarities just described for the pulvinus of *Hedyosmum* agree with those recorded for *Pilea* and other plants by Goebel.

DISCUSSION.

Evolutionary theory leads us to believe that the ancestors of the existing Chloranthaceae approached more nearly the normal or generalized angiosperm type, and therefore were without marked foliar sheaths and developed their lateral branches in the axil. This view is supported by the ontogeny of each individual node, as well as that of the plant as a whole, for in the earliest nodes of the seedling the sheath is inconsiderable and the lateral buds, which usually do not develop into branches, remain axillary. The writer holds that the evidence available supports the view that in the evolutionary sequence the development of the habit of extra-axillation *followed* the evolution of the sheath and was an effect of the presence of the latter, a result of the redistribution of organs made necessary by the mechanical conditions within the sheath, which was at the same time becoming higher and more massive. The removal of the pulvinus from the

foliar node is the result of the same readjustment. Its plasticity in regard to place of development at the present time, now at the base of the branches, now at the top of the sheath, lends weight to this theory. At the present time, the extra-axillation of the branches is more than is necessary to provide for mechanical equilibrium with the sheath—the habit, once initiated, has overshot the necessary end-point of readjustment.

It is interesting to compare the position of the branches with regard to the sheath in *Hedyosmum* with the situation in *Equisetum*, an old and conservative stock. In the latter, as is well known, the sheath becomes fused with the axis above the rudiments of the lateral branches, and the latter escape to the exterior by bursting through the overlying tissue. In the banana, on the other hand, where a very massive but open sheath is formed, the lateral bud, of undetermined morphological significance, develops on the side of the stem opposite the axil, between the free margins of the sheath. In its solution of the mechanical difficulty, *Equisetum* shows less plasticity than *Hedyosmum*; the banana, in the opinion of the writer, gives evidence of considerably more plasticity.

SUMMARY.

1. In *Hedyosmum arborescens* and related species a sheath, which surrounds the stem for a distance of about 15 mm. above each node, is formed by the connate bases of the opposite leaves.

2. In developmental sequence, this sheath pushes beyond the apical growing-point and surrounds it. The sheath is tightly closed above the bud, and affords the latter its only protection from the exterior.

3. The lateral branches spring from the shoot at some distance beyond the top of the sheath and, in the maximum example, 27 mm. above the axil of the leaf.

4. The lateral buds arise in the axil as in normal dicotyledons; their marked extra-axillation is secondary, and is brought about by the elongation of the axis in the region of their insertion upon the latter.

5. In weak shoots, where the lateral buds remain dormant, their extra-axillation is not sufficient to bring them to the top of the sheath.

6. Between the main lateral bud and the axil of the leaf there occurs an accessory bud, which remains dormant unless the principal bud above it is destroyed. The development of this bud is traced.

7. The sheath is provided with a well-developed collenchyma, and supports the stem during its period of intercalary growth.

8. The contact between the sheath and the stem, essential to the effective support of the latter, is made intimate by the development of periderm on the inner surface of the sheath.

9. A well-developed pulvinus occurs in the stem. In weak shoots this is

situated at the upper margin of the sheath, and accordingly in the central region of the internode. In strong shoots, the pulvinus is situated at or just above the level where the lateral branches become free.

10. The evolution of the habit of extra-axillation in the Chloranthaceae is regarded as causally related to the development of the massive foliar sheath.

ARLINGTON, MARYLAND,
March 16, 1927.

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DESCRIPTION OF PLATE XXX.

Illustrating the paper by Dr. A. F. Skutch on Peculiarities in the Structure of the Stem, related to the Leaf-sheath in *Hedyosmum*.

Fig. 1. Portion of a young shoot which has ceased to elongate, showing the sheath, the petioles inserted near its upper margin, and the lateral branches which have been carried above it by the intercalary growth of the node. This shoot, as well as those shown in Figs. 2 and 3, had somewhat wilted before the photograph was made. (Natural size.)

Fig. 2. Nodal region of a mature shoot. The main lateral branch to the left has been broken away, and its place has been taken by a shoot which has developed from the accessory bud. The accessory bud on the right side has developed only to a very slight degree. ($\times 1.5$.)

Fig. 3. A node from which the sheath has fallen, revealing the great degree of extra-axillation of the lateral branches. Note the scar left by the detachment of the sheath. (Natural size.)



SKUTCH — HEDYOSMUM.

Fig. 1.

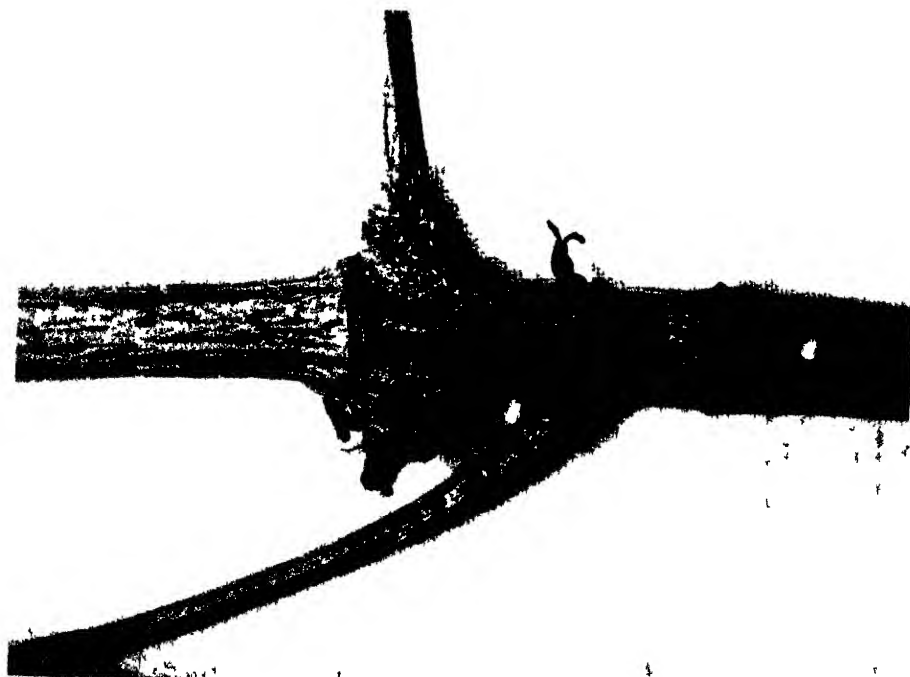


Fig. 2.

Observations on the Tubercles of *Ranunculus Ficaria*, L.

BY

A. C. HALKET.

With six Figures in the Text.

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I. INTRODUCTION.

FEW plants are vegetatively produced by the separation from the plant of specialized structures borne on their aerial parts. The most remarkable of these is perhaps *Ranunculus Ficaria*, L., the lesser celandine, which is reproduced by means of curious tuberous structures which are formed in the axils of the leaves. They are formed not only in the axils of the basal leaves of the plant but also in those of the upper cauline leaves of vigorously growing plants, even in those of the reduced leaves of the inflorescence axes. In the early part of the summer the aerial part of the plant withers and dies except for these little rounded bodies, which persist; these are freed by the decay of the rest of the shoot and become scattered; they remain dormant until growth begins again in the late autumn. They are produced so abundantly on plants growing luxuriantly under good conditions that they have given rise to the myth of 'potato rain' or 'rain of corn' (Kerner and Oliver (13)), and have been given the popular name

'Himmelsgerste' (Heaven's barley). This abundance makes the lesser celandine a troublesome weed in many gardens.

These tuberous structures were called 'tubercules'¹ by the early French investigators, and it seems advisable to employ 'tubercule' as a descriptive term, especially as the use of this term serves to emphasize the peculiar morphological nature of these structures, and to distinguish them from other vegetative reproductive bodies which occur in similar positions in certain other plants, such bodies, for example, as the bulbils of *Dentaria bulbifera*, &c.

The attention of the writer was attracted by the striking appearance of some flowering plants having these tubercles in the axils of the leaves of their flowering axes, and further interest was aroused on their closer examination. Finding variant descriptions given of these structures in the different text-books consulted, and finding also that they showed many features of interest, investigation was carried farther, and tubercles were examined at different periods during their development and growth. The tubercles occurring in the axils of the cauline leaves are very similar to the storage roots developed each year at the base of the stem, but, as much the greater part of the work was done with the tubercles, the account which follows deals with these structures. Observations made on the subterranean tuberous roots, as well as the similarity of their structure to that of the tubercles, lead one to suppose that the results obtained during the growth of the tubercles apply also to these storage roots.

II. MATERIAL.

Most of the plants used were obtained from two localities in the neighbourhood of Totteridge, Middlesex. The character of the soil differed in the two places, and the plants grew more luxuriantly in one with water-logged clay soil than in the other with comparatively dry soil containing a quantity of humus, but no difference was apparent in the occurrence or behaviour of the tubercles. Plants brought into the laboratory were kept in wet moss in a damp atmosphere. Most of the observations were made on fresh material.

III. MORPHOLOGY AND ANATOMY.

The plentifulness of production of the tubercles and the peculiarity of their appearance early attracted attention, their morphology interesting several of the French and German botanists. One of the first of these to

¹ John Lindley, in the glossary of 'The Elements of Botany', 1847, p. xcvi, defines 'tubercules' as 'simple roots which acquire a succulent condition, become reservoirs of vegetable food and serve for propagation, in consequence of being terminated by a bud', and illustrates his definition by a drawing of the underground parts of a terrestrial orchid.

whom reference has been found was Payer (18), who in 1846 described the tubercles as 'des racines de bourgeons'. During the years following, accounts were given by Oschatz (17), Irmisch (9, 10 and 11), Aimé Henry (8), Clos (2), Germain de Saint-Pierre (6), van Tieghem (28), and others, some of whom consider a tubercle to consist of the swollen axis of an axillary bud, but most describe it as a root structure formed in connexion with the bud. The mode of origin, the form of the growing-point, the relation to the bud, and the external form were the criteria used to determine the morphology, van Tieghem (28) being the only one to give details of anatomical structure, though the presence of a central vascular strand in the tubercle is mentioned by Clos (2), commented on and figured diagrammatically by Henry (8), and Irmisch (10, 11).

The tuberous structures occur singly or in groups of two or three in the axils of the leaves; on some stems, the terminal bud not developing, they appear to arise on the stem apices, though they are actually formed in the axils of the two uppermost leaves. The simplest structures are found in the axils of the leaves on the flowering axes of vigorous plants, and the mode of formation of the tubercle is seen most easily if these are examined.

When the flower is still unopened a bud can be seen in the axil of each leaf on its axis; the buds develop and form the tubercles. The first leaf of the bud arises on its adaxial side, and, as a rule, develops so far that its lamina can be distinguished as well as its sheathing base. A little prominence arises on the abaxial side of the bud, i.e. on the opposite side to the leaf, out of which a structure emerges which soon increases in size, the increase being quite disproportionate to the increase in size of the bud itself. This structure becomes the tuberous part of the tubercle. Fig. 1 shows a flowering axis, A, with two developing tubercles, B and C, from the axils of its leaves, *b* and *c*. When mature, a tiny bud is seen attached to one end of the smooth ovoid structure which has arisen from it, as is shown in Fig. 1, D. In some cases the first leaf of the bud develops farther and becomes a small foliage leaf, as is shown in Fig. 1, C. As the tubercles increase in size rudimentary buds may arise in the axils of the leaves of the axillary bud, so that more than one bud can be seen on the mature tubercle. In some cases, too, one or more additional tuberous structures may arise from an axillary bud, and groups of two or three of these are found in the leaf axils. Van Tieghem considers that a definite relationship exists between the rudimentary buds and the additional tuberous bodies, each of which, he says, is formed in connexion with, and at the base of, a bud. His contention receives support from the fact that, when tuberous bodies of an axillary group are separated, one carries the main bud while each of the others bears at least one tiny bud which can only be distinguished on careful examination; but to confirm his statement would require further and much more detailed work on the development of the tubercles.

The mode of origin of the tuberous bodies and their lack of lateral appendages suggest that they are morphologically roots, and this interpretation is confirmed by their anatomical structure, which is that of a root.

Van Tieghem (loc. cit., p. 93) describes the anatomy of the mature storage roots of the adult plant and states that the organization of the

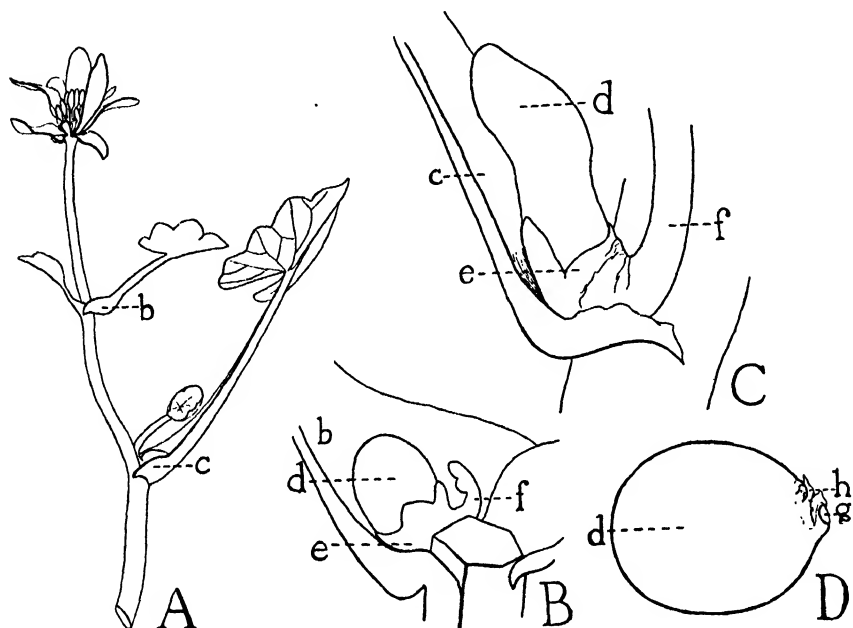


FIG. 1. A. Flowering axis bearing tubercles in the axils of the leaves *b* and *c*. Nat. size. B. Young tubercle in the axil of leaf *b*, showing the endogenous origin of the adventitious root. $\times 7.5$ approximately. C. Older tubercle in the axil of leaf *c*. $\times 6$ approximately. D. Mature tubercle showing one bud. $\times 6$. Root hairs omitted. *d* = adventitious root; *e* = tissue of bud; *f* = first leaf of bud; *g* = place of attachment; *h* = bud.

tubercles is in all respects similar. His description is as follows: 'L'axe en est occupé par un cylindre de cellules étroites et longues sans fécule, au milieu duquel sont rangés en cercle cinq faisceaux (quelquefois quatre) vasculaires, formés de vaisseaux rayés et spiralés sans trachées déroulables. Ce cylindre étroit est entouré d'une gaine fort épaisse de grandes cellules polyédriques, gonflées de gros grains d'amidon en général simples, et dont le diamètre atteint 0.040 mm.; cette couche est elle-même revêtue d'une sorte d'épiderme formé de deux rangées de cellules à paroi jaunâtre, sans fécule; les cellules de la couche externe sont plus aplaties que les autres, et un grand nombre d'entre elles se prolongent en longs poils unicellulaires. Le faisceau central règne dans toute la longueur de la racine, jusqu'à son extrémité un peu ombiliquée, où il se perd dans le tissu amylacé, et où l'épiderme possède la même structure qu'ailleurs, sans qu'on puisse y distinguer de pilorhize quand le tubercle est entièrement développé.'

This account, in fact, gives an adequate description of the more obvious anatomical characters of the root of the tubercule, but not of the ending of the stele, and it omits several points of interest.

The number of alternating xylem and phloem groups seen in the small central cylinder varied from two to six, four or five being the more usual number. The xylem strands are small and consist of a few reticulate elements, short vessels and tracheides, those of the protoxylem being in

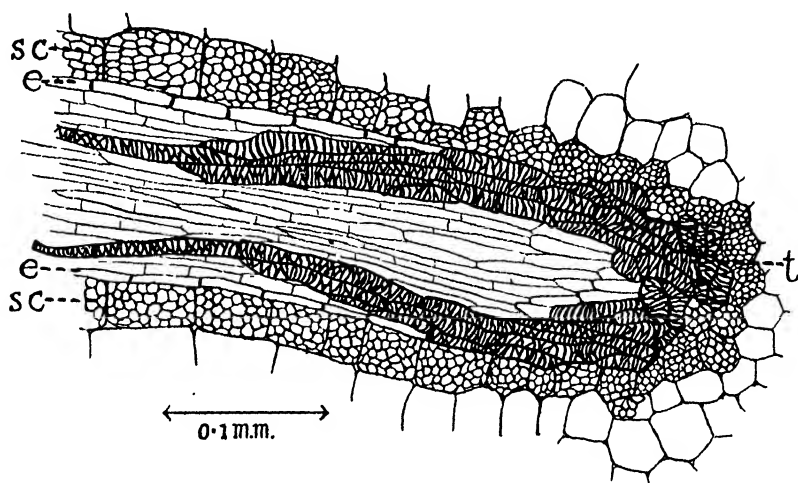


FIG. 2. Longitudinal section of the terminal portion of the stele of the tuberous root. *e.* = endodermis; *s.c.* = storage cells of cortex; *t.* = terminal group of tracheides. Drawn with the aid of a projection prism.

most cases distinguished only by their smaller diameter. In the majority of tubercles these strands increase in size towards the base and end in a group of short reticulate tracheides (Fig. 2), many of which are developed from cells cut off from a rudimentary cambium.

The central stele is surrounded by an endodermis, whose cells show the characteristic Casparian strip on their radial and transverse walls. In the mature tubercule this endodermis continues almost the entire length of the stele, but does not completely enclose it, as the differentiated endodermis stops at the terminal group of tracheides (Fig. 2). These tracheides are thus in direct contact with the storage cells.

The cortex in which the starch is stored consists of somewhat regularly arranged parenchymatous cells with small intercellular spaces. The structure of these cells recalls that which is characteristic of the specialized storage tissue of the endosperm of certain seeds. In the mature tubercule the cellulose walls are somewhat thick, and have numerous simple pits irregularly distributed on the parts of the wall in contact with the adjacent cells (as is shown in Fig. 3, A). These walls absorb water very readily when dry; sections of dried tubercles placed in water rapidly expanded, the moist

condition being about 50 per cent. larger than the dry. The protoplasts of these cells are connected by many fine protoplasmic strands (plasmodesmen) which pass through their walls (Fig. 3, B).

The cells of the two external layers, described by van Tieghem as 'une sorte d'épiderme', are differentiated from the others (Fig. 4, F). In the mature tubercule, as he describes, their walls are slightly yellow and they contain no starch.

In the young tubercule, while it is still protected by the base of the

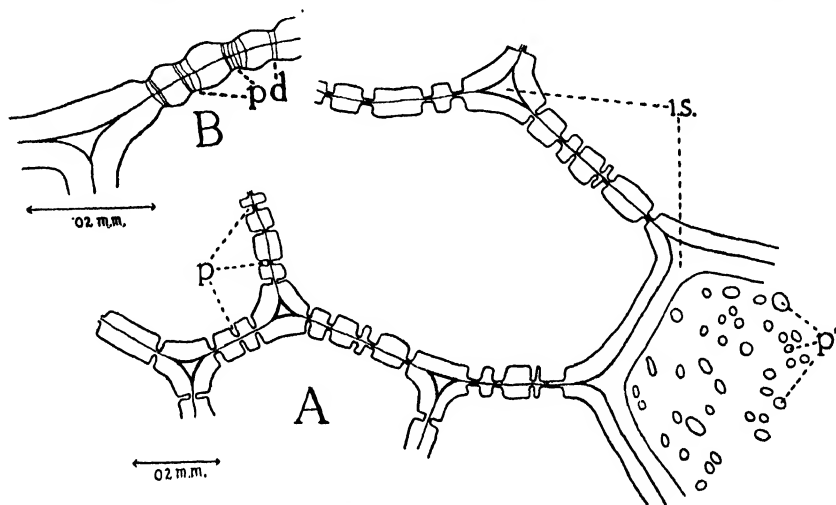


FIG. 3. A. Walls of the cortical cells of the storage root. *p*. = pits in section; *p*'. = pits in surface view; *i.s.* = intercellular space. B. Wall of cortical cell treated with sulphuric acid and iodine, and stained with gentian violet. *pd.* = plasmodesmen. Drawn with the aid of a Zeiss-Abbe camera lucida.

leaf in whose axil it is developing, the walls of the cells of the two peripheral layers are of cellulose and are not differentiated from the others. The outermost layer can be regarded as a true piliferous layer, many of the cells being prolonged into hairs. These hairs may absorb water at this stage as they have cellulose walls. They are produced most abundantly on plants growing in damp places. As the tubercule develops the walls of the cells of these two layers gradually become cuticularized, those of the epidermal layer (including the root hairs) becoming so first. The cell walls of the subepidermal layer then become cuticularized, their outer walls being altered before their inner ones. Also some of these cells give the cutin reactions at an earlier stage than their neighbours, so that this layer becomes cuticularized irregularly. In addition, during development, the cells of this subepidermal layer become differentiated, and in the mature tubercule two kinds can be recognized. The majority of the cells are cuticularized on all their walls. Interspersed with these are slightly smaller cells which have their external walls thickened; these

thickened walls are cuticularized, but the inner walls are not so. These cells are also distinguished by their contents: they contain larger nuclei and more protoplasm than the other cells (Fig. 4, E and F). This differentiation

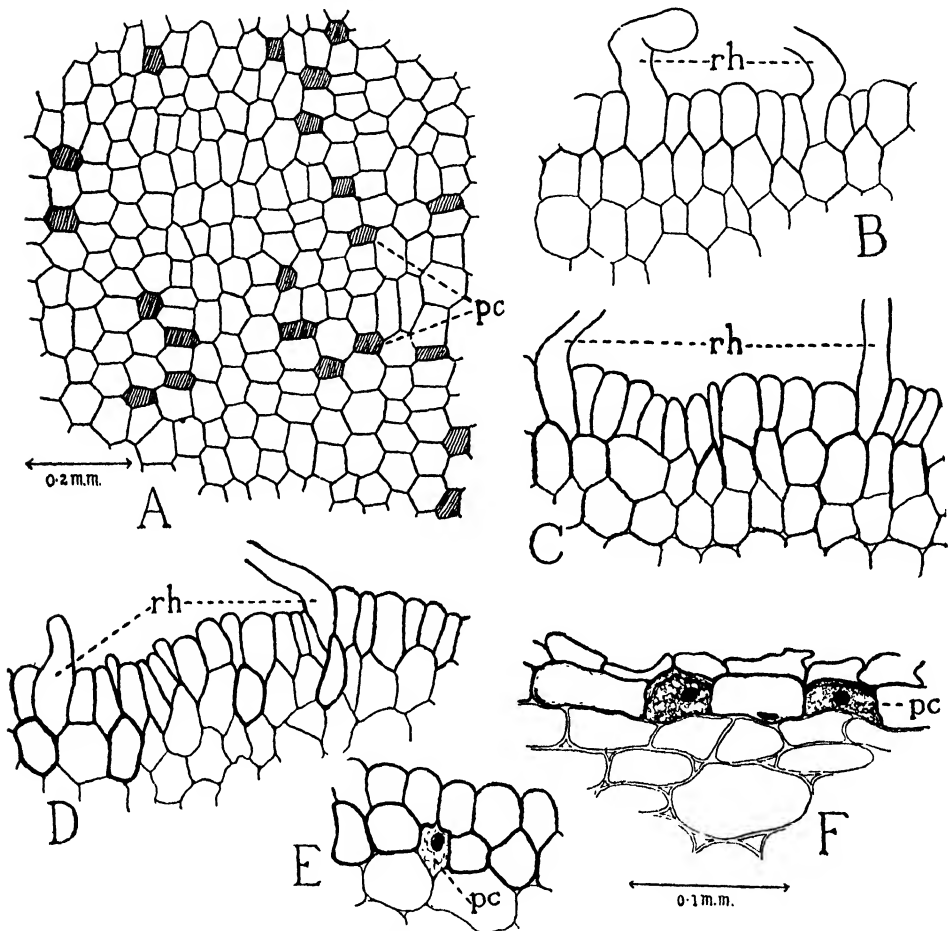


FIG. 4. Peripheral layers of the storage root of the tubercle. A. Surface view of the exodermis. The 'passage cells' are shaded. Drawn with the aid of a projection prism. B, C, D, E, and F. Peripheral layers from tubercles at successive stages of development as seen in transverse section. The cuticularized walls are drawn with a thick line; those with the thickest line stained most deeply with Soudan III. The width of the line does not denote thickness of wall. *p.c.* = 'passage cell'; *r.h.* = root hair. Drawn with the aid of a Zeiss-Abbe camera lucida.

into cells of two kinds recalls that found in the exodermis of the roots of certain Javanese plants, e.g. *Rauwolfia javanica*, described by Janse¹ (12).

The gradual cutination of the cells of the two peripheral layers and the differentiation of the cells of the exodermis are shown in Fig. 4. These cuticularized layers form the protective tissue of the tubercle: the exo-

¹ These smaller cells in the plants described by Janse were called by him 'cellules de passage', as he found that the mycorrhizal fungus, if present, always entered the root by penetrating these cells.

dermis is the true protective layer, for the cells of the piliferous layer, stretched during growth, often become separated from each other.

For a short time after appearing the root of the tubercule grows in length by division of its apical cells, and while growth is continuing a small root cap can be distinguished protecting its apex. Growth in length soon ceases, but increase in thickness, due to the enlargement of the individual cortical cells, continues for some time, until the cortical cells are filled with starch. During this process the root cap becomes stretched, and in the adult stage can no longer be distinguished.

Thus the vegetative reproductive structures of *Ranunculus Ficaria* consist of axillary buds which have each developed one or more adventitious tuberous roots. The buds remain small, and much the greater part of the tubercule consists of the enlarged adventitious root, in whose cortical cells starch, the reserve food material, is stored. The root has a well-protected surface and a specialized storage tissue.

IV. GROWTH OF THE TUBERCULES.

These small specialized structures are, like most bulbs, corms, &c., resistant to adverse conditions; they grow after being dried and after being frozen. The bud is minute and its growing-point is well protected by its sheathing scale leaves.

Observations of the growth of tubercules have been made for three seasons, and it was found that the time when growth begins and the rate of growth vary with the individual and the season. In some cases buds begin to grow early in the autumn, while others start growing later, so that some tubercules with small buds can be found in January.

The bud elongates a little, then two, three, or more adventitious roots arise at its base; these are the ordinary absorbing roots (Fig. 5, A). After the growth of these absorbing roots the bud grows more rapidly, and two or three scale leaves develop, followed by one or more foliage leaves.

About March an obvious swelling appears at the base of the short stem, and out of this a thicker root, which becomes a new tuberous storage root, emerges (Fig. 5, C and D). In most vigorously growing plants more than one tuberous root may be formed.

All these 'first-year' plants remain small, and, judging by the amount of growth made during the first growing season, several years must elapse before the plants flower.

While this growth is going on, the cells of the old tuberous root of the tubercule are being depleted of their contents, and the root becomes contracted and wrinkled (Fig. 5, D and E). This old root finally decays, the leaves wither, and, at the close of the plant's vegetative season (the end of May or the beginning of June), the bud with its new adventitious tuberous root, or roots, remains and lies dormant during the summer.

The tuberous roots are storage organs, and as the walls of the root hairs and of the other cells of the external layers are cuticularized when mature, one would expect them to absorb little or no water. To ascertain the truth of this supposition, growing plants were placed in dilute solutions

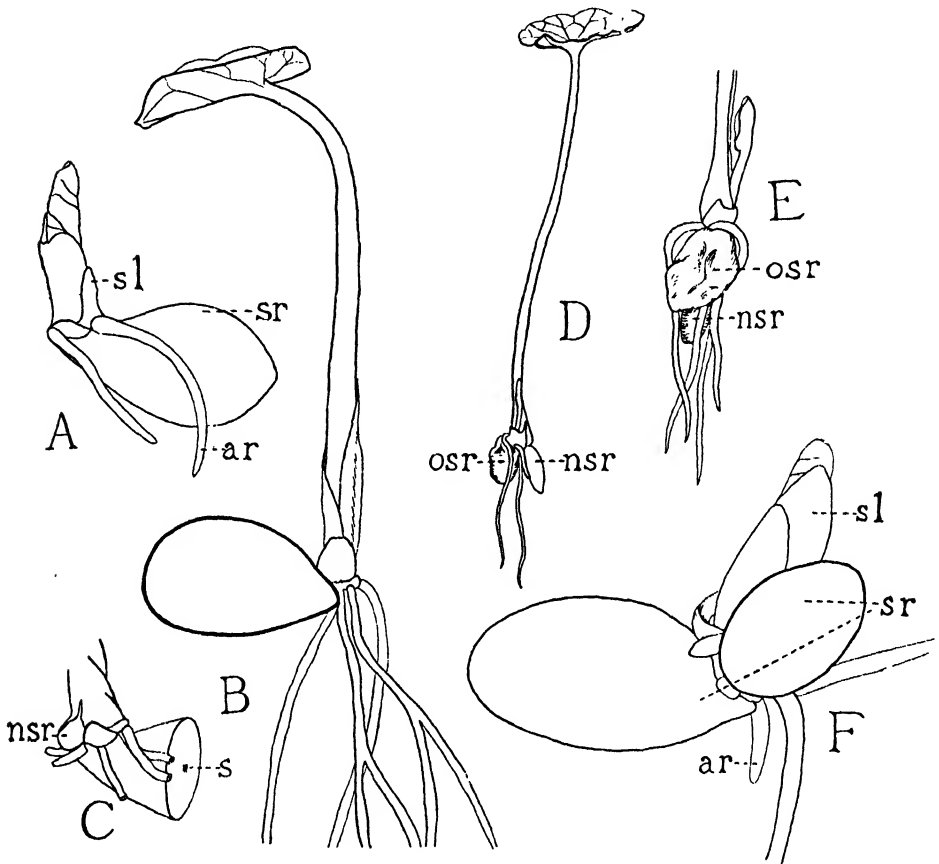


FIG. 5. A, B, C, and D. Tubercles at successive stages of growth. A $\times 3.75$; B $\times 4$; C $\times 3$; D nat. size. E. Another view of D. $\times 2$. F. Tubercle with two adventitious storage roots. $\times 5.5$. s.l. = scale leaf; s.r. = storage root; a.r. = absorbing root; s. = stele of storage root; o.s.r. = old storage root; n.s.r. = new storage root.

of eosin or of methyl green. Plants with their tuberous roots partly in the solution had the walls of their external cells stained, and in some cases the walls and contents of the 'passage cells' of the subepidermal layer were stained also. When the plants were left several hours in the eosin solution, the cortical cells adjacent to the passage cells were sometimes faintly coloured also. It may therefore be concluded that very little water is absorbed by these swollen roots, and that the greater part of that absorbed enters through the specialized cells in the subepidermal layer.

V. THE DEPOSITION AND REMOVAL OF STARCH.

i. *Deposition.* During the development of the tubercle, starch is deposited in the cortical cells of its root. The cells of the two outermost layers and those of the endodermis remain practically free from starch, though small grains are sometimes found in the cells of the endodermis. The cells of the stele also remain practically free from starch. Deposition starts at an early stage, and shortly after the tuberous root appears minute grains, staining blue with iodine, can be seen in the cortical cells. They are seen first in the cells at the bud end adjacent to the stele, and gradually increase in size in these cells, while small grains appear in the neighbouring cells. This process continues and starch is deposited in the cells from the bud end downwards, and from the stele outwards. The cells at the root apex (though not of the root cap itself) and those of the peripheral layers are the last in which starch grains are produced.

The course of deposition agrees with the supposition that the sugar from the leaf is brought to the axillary bud with its developing root, and diffuses slowly out from the stele of this root through the endodermis to the adjacent cortical cells, where the leucoplasts transform part into starch, while part diffuses into the further cortical cells, in which starch grains appear in due course. During this process the concentration of reducing sugars in the stele is, as one would expect, greater than in the surrounding cortex, for in thick longitudinal sections treated with Fehling's solution the stele becomes red, while the rest of the tissue remains colourless.

The deposition of starch begins when the cells are quite young and are full of protoplasm with only small vacuoles. The grains appear scattered throughout the protoplasm; they increase in size gradually, surrounded by the protoplasm, till the cells have grown to their full size. When the cells are mature they are full of ovoid starch grains enclosed in meshes of protoplasm. The nucleus occupies a central position in the cell and, as a rule, is spherical, though it has been seen lobed in some cells while deposition is proceeding. In mature cells it seems to be somewhat crushed by the starch grains and often becomes angular through their pressure.

ii. *Removal.* Starch disappears from the cells during growth; the process of removal is slow and continues for some time. Examination of a large number of tubercles at different stages of growth showed that the starch was removed from the cortex in a definite manner. Starch is hydrolysed in all the cells, but its removal is not uniform over the whole cortex; it takes place more quickly from certain zones. The central part of the cortex is cleared first; the cells there become empty of starch, while those nearer either of the two ends and those nearer the stele and the periphery continue to contain starch. The depleted region gradually becomes larger as the starch is removed from the adjacent cells, until all the cortical cells

are cleared, the last to be freed being those abutting on the endodermis and on the suberized external layers of cells. The effect of 'zonation' thus produced in the cortex is very striking at certain states of depletion.

At first no explanation of this regional removal of the starch could be found. Apparently it might be due to one or more of the following causes :

1. *The greater metabolic activity of cells in the central region.* This found some support from certain observations made on tuberous roots separated from their main bud. It has been mentioned earlier that some of the axillary buds have more than one tuberous root, and that very rudimentary buds are developed in the axils of their first leaves. Tubercles with two roots were taken at the beginning of June 1924, and the roots separated; one from each tubercle was found to retain the main bud, while the other had one or more of the minute secondary buds. Several of the latter were kept in damp moss to see if they would grow; they remained alive and healthy, but no visible development took place in the following autumn, and it was not until nearly the end of January that the small buds had grown large enough to be seen without a lens. Sections of the tuberous roots were then cut, and it was found that all the cortical cells had lost some starch, while those of the central region had lost all their starch. This starch was almost certainly used in respiration, it was not all used for growth. It is therefore possible that the central cortical cells are more vigorous than the others, and so get rid of their starch more quickly.

2. *The greater production of diastase in the central cortical cells.* That diastase is formed in the tuberous roots can be shown easily by squeezing out some sap and allowing it to act on a film of starch paste. An attempt was made to determine whether any difference in the amount of enzyme present in the various regions of the cortex could be demonstrated. Drops, equal in size, of very thin starch paste¹ were placed on slides and dried. Tubercles were cut transversely and placed with their cut surfaces in contact with the starch film; they were then left in a saturated atmosphere. After an interval the pieces of tubercle were removed from the starch, and the film was dipped in a solution of iodine and examined microscopically; also, the pieces of tubercle were examined to see if any of the film had adhered to the cut surfaces and so been removed.

In successful experiments, when the action had continued for some time, a rough print of the section was found on the film. This print had been produced by the action of the diastase present in the cells of the tubercle. With any tubercle the amount of starch removed from the film depended on the time contact lasted, and if this time was sufficiently

¹ The paste used was made from potato starch in the usual way, but to obtain a homogeneous film it was found necessary to filter the paste to separate off the 'skeletons' of the grains.

long, all the starch underneath the piece of tubercle would be hydrolysed and no print formed.

Many tubercles, at various stages of growth, were examined in this way without finding any difference in the various regions of the cortex ; as far as could be shown by this method, diastatic activity was approximately equal in the cells of all parts of the cortex.

3. *The more rapid diffusion from the central region.* If the products of the hydrolysis of the starch diffused equally quickly from all the cells in the direction of the developing bud, one would expect the starch to disappear first from the peripheral cells, and from those at the lower end of the tubercle, and that it would remain longest in the cells adjacent to the stele and those at the upper end near the growing bud. As this was observed not to be the case, experiments were made to determine the path taken by the sugar in passing from the cortical cells to the growing bud. The sugar in solution might travel only through the cortical cells themselves, in which case it could pass through the walls as well as through the protoplasts, or it might travel by means of the tissues in the stele, in which case passage through the walls, except at the extreme base, would be hindered, if not stopped, by the narrow Casparian strip on the endodermal walls (de Lavison (14) and Priestley and North (19)).

It seemed possible to determine the way traversed by the sugar if an experiment were so arranged that only one of these paths was open. Accordingly some tubercles were taken and cuts¹ were made through the cortical tissue of the tuberous roots. The cuts were made either parallel or perpendicular to the stele. The cut surfaces were isolated from each other by placing a piece of thin glass (part of a cover-slip) in the cut. The plants were then left to grow farther. After an interval the distribution of the starch was determined by means of longitudinal sections. It was found, when the roots were partly depleted of starch, that in those cut longitudinally considerably more starch was present in the part of the cortex separated from the stele than in the other part, while in those cut transversely little or no difference could be seen in the amount of starch above or below the cut, but more starch was present on the cut side than on the uncut. However, when cut tubercles are left to grow for a longer time, depletion is carried farther and the starch disappears from all the cells. From the results so obtained it is concluded that the sugar passes away mainly through the stele, but that some travels by way of the cortex.

The main current of diffusion being towards the stele would explain

¹ The cut surface became protected by a brownish deposit of a fatty nature, probably suberin, which stained red with Sudan III and yellow with chlor-zinc-iodine solution. This substance was found deposited on the walls of the cells bordering on the cut, and also in the intercellular spaces in its neighbourhood. Cells with these impregnated walls remain full of starch, while those immediately below are empty. No trace of periderm was found.

the persistence of the starch in the tissue adjacent to it, for in these cells the concentration of sugar would be maintained by that coming from the cells farther out. It is more difficult, however, to account for the persistence of the starch in the peripheral zone. This may be due to a difference in the nature of the cell-wall, as Wood (30) found that in the tuberous root of *Ranunculus Ficaria* the walls of the three rows of cells below the piliferous layer became dark brown when subjected to the chloramine test, while the others remained colourless. Also the cells are smaller at the periphery, and the greater number of cell-walls to be traversed may decrease the rate of diffusion.

From the evidence derived from a consideration of these three possibilities, it would appear that the difference in the amount of starch present in the various regions of the cortex may be caused by a slower rate of diffusion of sugar from the peripheral zone, and by the possibly greater respiratory activity of the central cells, and not by any appreciable difference in the amount of diastase present in the cells.

VI. CYTOLOGICAL CHANGES IN THE CORTICAL CELLS DURING DEPLETION.

When the tubercule begins to grow the storage cells are crowded with starch grains embedded in protoplasm and surrounding the nucleus, which is in the centre of the cell.

The changes that take place during growth were determined by examining and comparing numerous tubercules at different stages of development. It was found that the contents of the storage cells show a definite series of changes due to the gradual disappearance of their contents as growth proceeds. These changes in the contents at different stages in the depletion of the cortical cells are shown in Fig. 6. The drawings were made from intact cells in sections of fresh tubercules mounted in saline solution (0.75 per cent. NaCl) to which a few drops of an iodine solution were added. It will be seen that the starch first disappears gradually, then the protoplasm, until, in the final stages, the cells are empty, all the contents having been utilized.

It is simplest to follow the fate of the different constituents of the cell separately.

i. *Starch grains.* As the starch is hydrolysed the grains become smaller gradually; hydrolysis takes place evenly over the surface, for no 'etching' of the grains was observed such as occurs in those from the endosperm of germinating wheat or barley seed. While the starch grains become smaller they remain crowded together, massed in a bunch round the nucleus. The cluster of grains is large in the early stages (Fig. 6, B and C), but as growth continues each grain, and therefore the whole central mass,

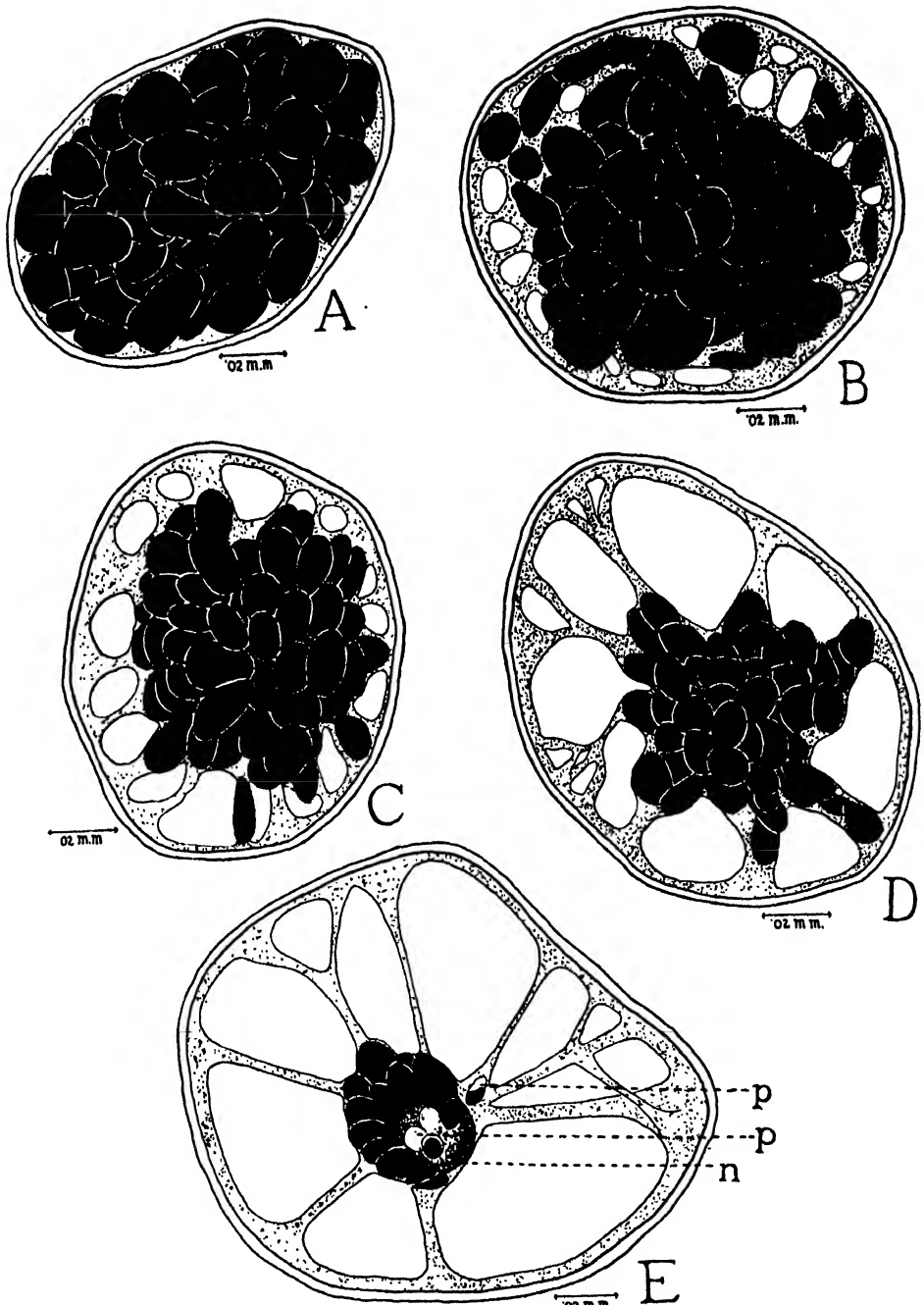


FIG. 6. Series of drawings, made from intact cortical cells from transverse sections of fresh material, illustrating the changes that take place in the cells during the growth of the tubercule. A-H. Storage cells from tubercules at successive stages of growth, showing the gradual removal of all the cell contents as growth proceeds. A-E. Gradual disappearance of starch. In the cells the black masses represent starch, the dotted areas cytoplasm, and the clear areas vacuoles. *n*. = nucleus; *p*. = plastid. The pits in the cell-walls are not shown.

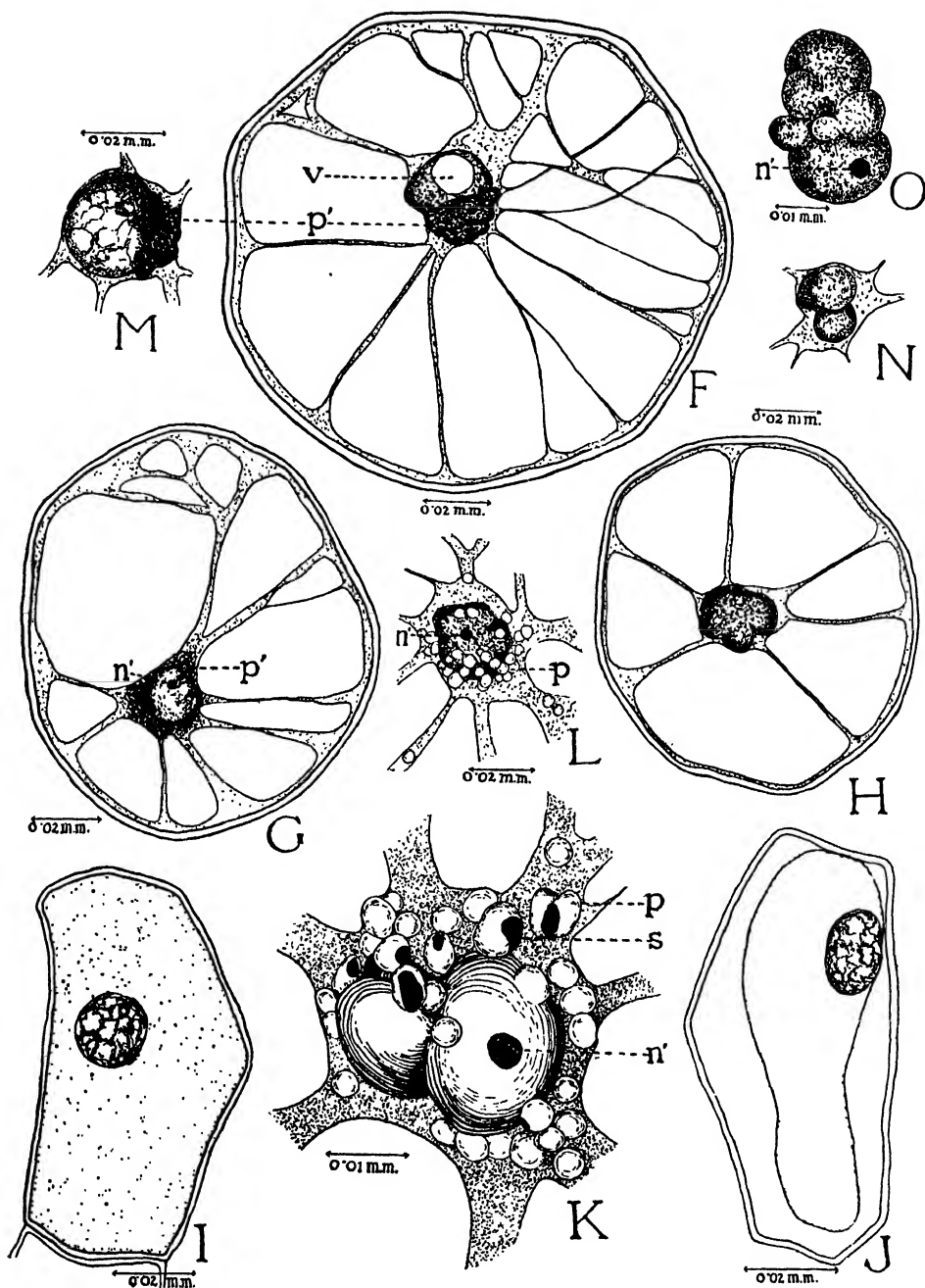


FIG. 6 F-O. F-H. Degeneration of plastids and gradual disappearance of protoplasm. I and J. Cells in which nearly all the contents have disappeared. I. In surface view. J. Plasmolysed cell seen in section. K. Lobed nucleus surrounded by plastids, some with starch still present. L. Lobed nucleus surrounded by plastids. M. Nucleus and degenerating plastids from cell similar to F. N and O. Lobed nuclei from cells similar to H. n'. = nucleolus; p. = plastids; p'. = remains of plastids; s. = starch; v. = vacuole in nucleus. The drawings in Fig. 6 were made with the aid of a Zeiss-Abbe camera lucida.

becomes smaller, until finally no starch is left (Fig. 6, D, E, and F). It is very noticeable that though the grains decrease in size they still remain clustered round the nucleus. This close contact may be brought about by the movement of the protoplasm, for in the living cells of almost all sections examined very active rotation and circulation of the protoplasm was observed, though no movement of the starch grains could be detected.

ii. *Plastids*. After most of the starch had disappeared from the cells, small circular bodies, which stained yellow with iodine, were noticed around the nucleus (Fig. 6, L), and careful search revealed that some of these had fragments of starch attached to them (Fig. 6, E and K). It is concluded, therefore, that these small protein bodies are the amyloplastids which persist after the starch is removed. These plastids can be distinguished as entities for a brief period only: soon they become massed together and their outlines blurred (Fig. 6, F and M). Then all trace of the individual units disappears and the nucleus is seen surrounded, or partially surrounded, by a dense layer of protein (Fig. 6, G). This gradually becomes less well defined until it can no longer be distinguished, and the nucleus remains surrounded by the undifferentiated cytoplasm of the cell (Fig. 6, H).

Considerable uncertainty prevails as to the fate of an amyloplastid after the formation of a large starch grain such as is formed in specialized storage tissues. Lately, Eames and MacDaniels (4), summing up the position in their recent text-book, say, 'It is not known with certainty whether, when a grain is mature, it is freed from the plastid, or is still surrounded by a very delicate layer of plastid substance'.

The plastids appear to behave differently in different plants, for Alvarado, according to Léon Dufour (3), finds: 'La transformation du mitochondre ou du leucoplaste en un grain d'amidon a lieu totalement et on ne constate jamais la formation d'une vésicule quelconque'; while Likoue Tchang (26), examining the behaviour of starch grains in the cotyledons of the haricot bean and the pea, finds the plastid absent in some cases and present in others; he reports that in the haricot the large simple grains of starch disappear and leave no trace, but that in the pea two kinds of grain can be distinguished, one behaving like the grains in the haricot, the other regenerating chloroplasts. Also Emberger (5) finds that differentiated plastids persist, as he states, in a paper reporting the results of observations made more particularly on the roots and leaves of asphodel (*A. cerasifer*, L.) and the bulb of the white lily (*Lilium candidum*), that differentiated plastids, when the 'produits d'élaboration' disappear, break up into a number of pieces which subdivide, till finally 'les gros plastes sont revenus à l'état mitochondrial'.

In *Ranunculus Ficaria* the amyloplastids persist after the formation of the storage grains, for, though they were not distinguished in the fully formed grains, when the greater part of the starch is hydrolysed they become

apparent with the remnant of starch attached, and are seen as little circular bodies after all the starch has disappeared. These plastids, however, neither subdivide and return to the mitochondrial state, nor do they long survive, but they become merged in the general cytoplasm of the cell and so disappear.

iii. *Nucleus*. As mentioned above, the nuclei in the cortical cells of the mature tubercule often become angular through pressure from the surrounding starch grains. Examination of many tubercules at different stages of growth led to the conclusion that many nuclei alter in shape and become lobed while the starch is being removed. The amount of lobing varies, but seems to increase as digestion proceeds, for the nuclei of the depleted cells are more lobed than those in partially emptied cells (Fig. 6, G, H, K, and L). These lobed nuclei are large and contain as a rule one, but occasionally two or three, conspicuous nucleoli. In some cells the lobing at some stage had evidently been carried farther, and resulted in the division of the nucleus, for frequently two nuclei were observed in one cell. In one exceptional case five nuclei were seen in one of the cells.

In the later stages of the process the nucleoplasm appears less homogeneous, and some of the nuclei develop large vacuoles (Fig. 6, F). It would appear that after the cells are depleted of starch the nuclei change again in shape and resume their spherical form, for when the tubercules are wrinkled all the nuclei are seen to be rounded, and they also look less dense in structure (Fig. 6, I and J). Later still, the nuclei appear structureless and they eventually disappear.

The lobed form and large size of the nucleus make it a very striking feature in the cell, and one is led to inquire whether this lobing is characteristic of the nuclei of storage cells in general and due to the metabolic¹ processes taking place in the cells.

This question must be left unanswered until further investigation has been made. The results of cursory examination of the scale leaves of the bulbs of tulip and snowdrop and of the cotyledons of the seedlings of the broad bean, the pea, and the scarlet runner, which revealed lobed nuclei in some of the cells, suggest, however, that they may frequently be present in storage tissues from which the food reserves are being removed, though no records of their occurrence were found in the literature consulted.

It is well known that the lobed or (as it has been called) 'amoeboid' form of the nucleus is characteristic of some cells in which very active metabolic changes are taking place. Records of their occurrence are numerous, but only brief mention of them can be made here.

Guilliermond (7), investigating the cytology of the seeds of some of the Gramineae, found that in the resting stage and during all the time of germi-

¹ The term 'metabolism' is used in its widest sense to include all the chemical changes taking place in the cell.

nation, the nucleus of most of the cells of the embryo presented a most characteristic irregular contour. He says, 'Il est divisé en plusieurs lobes saillants, ce qui semble témoigner de la grande activité de ces cellules', but gives no figures.

Enlarged and lobed nuclei have, perhaps, been recorded most frequently as occurring in the mycorrhiza of many plants. They have been described by many workers on this subject, some of whom have given drawings of the nuclei. Of the earlier records in which these nuclei are described and figured, the following may be cited: Janse (12) in *Lecanorchis javanica*, Magnus (15) in *Neottia Nidus-avis*, and Shibata (24) in *Alnus*. Of the records, giving drawings, of recent date mention may be made of that of Rayner (20) in *Calluna vulgaris*, and of McLennan (16) in *Lolium temulentum*. In all these plants the lobed nuclei were found in the cortical cells of the root infected by the mycorrhizal fungus in which active metabolic changes leading to the destruction of the fungus were taking place. These investigators associate the alteration of form of the nucleus with the active metabolism going on in the cells or with the abundance of nutritive substances present through the 'digestion' of the mycorrhizal fungus.

Nuclei become lobed also in the cells of certain specialized tissues. Torrey (27) and Reed (21) have noted their presence in the cells of the epithelial layer of the scutellum of *Zea Mays* at an early stage in the germination of the seed; but Guilliermond (7), investigating the cytological changes in the epidermal cells of the scutellum of various grasses, found that the nuclei in the cells of *Hordeum* and of *Triticum* did not change appreciably—they elongated a little but did not become irregular in contour—and that those of *Zea Mays* altered in form but did not become markedly lobed.

Schniewind-Thies (23), investigating the structure of the nectaries of certain monocotyledonous plants, found the nuclei of the secretory cells of *Funkia* and *Hemerocallis* variously indented, lobed, incised, and branched. This change in nuclear shape, however, cannot at present be said to be a characteristic feature of nectar-secreting cells, for Schniewind-Thies did not find it in all the plants he examined, Saunders (22) does not mention it when describing the septal glands of *Kniphofia*, neither did Stockard (25) observe amoeboid changes in the nuclei of the cells of the secreting layer in the nectar glands on the stipules of *Vicia Faba*.

A few instances of the occurrence of alteration of form of the nucleus, referred to as 'lobing', have been selected for mention here, but further records could be given of their presence being noted in other cells in which active metabolic changes are taking place. In the cases quoted, as in other cases besides, the cells in which lobing of the nucleus occurs are distinguished by the abundance of the nutritive material present, and, in most cases, by the presence of an enzyme proved or suspected.

The data at present available do not allow the correlation of the

change of shape of the nucleus with either of these two factors, the abundance of nutritive material or the secretion of the enzyme, for both are present in the cell at the same time. However, the data are sufficient to justify the relating of change in nuclear form to intense metabolic activity.¹

iv. *Cytoplasm*. Very little cytoplasm is visible in the storage cells when the tubercle is mature; the cells are packed with starch grains, the cytoplasm occupying the interstices between the grains. At this stage no vacuoles were detected in the protoplasm. After the hydrolysis of the starch has commenced and the starch grains have become smaller, the cytoplasm becomes more obvious at the periphery of the cell and vacuoles appear in it. As hydrolysis continues, the vacuoles increase in size, so that the cytoplasm becomes separated into a peripheral layer and numerous strands which connect this layer with the cytoplasm surrounding the central cluster of starch grains (Fig. 6, B and C). As depletion proceeds and the cluster of grains becomes smaller, the vacuoles increase in size, while the cytoplasmic strands and the peripheral layer become thinner (Fig. 6, D and E).

The enlargement of the vacuoles and the diminution of the cytoplasmic strands continue (Fig. 6, F and G) until when all the starch has disappeared the nucleus is seen suspended in the centre of the cell by a number of very thin cytoplasmic strands connecting it to the almost equally thin protoplasmic membrane (Fig. 6, H). The protoplasm continues to disappear, the connecting strands become more attenuated, then break and allow the nucleus to fall on to the cell wall, as is shown in the last stage figured (Fig. 6, I). At this stage the cytoplasmic lining has become so thin that it can only be distinguished through the presence of the small oil drops, unless the cell is plasmolysed, when it becomes evident, as is shown in Fig. 6, J. The disappearance of the protoplasm continues farther until there is none left and the cells are empty. The central cells lose all their protoplasm first, and those abutting on the stele retain it longest. By this time the tubercles have become wrinkled, most of the water being absorbed with the other contents of the cells.

As mentioned earlier, circulation of the protoplasm occurs, and it can be observed in the cortical cells at all stages of depletion from the appearance of the vacuoles to the breaking down of the cytoplasmic strands. The movement is very active and the presence of numerous oil drops in the cytoplasm makes it easy to observe.

The occurrence of this active movement of the cytoplasm is interesting in view of the opinion expressed by de Vries (29), that the circulation of the protoplasm greatly facilitates the distribution of substances in the cell and their transport from cell to cell.

¹ Several investigators have recorded the presence of lobed nuclei in the cells of various tissues, and have regarded them as stages in the amitotic division of the nucleus.

v. *Oil.* No oil was detected in the mature tubercles, but shortly after growth started, at an early stage in the hydrolysis of the starch, numerous small drops were seen in the protoplasm. These drops gave the usual reactions for oil, staining red with Scharlach R and with Soudan III and dark brown with osmic acid. The amount of oil in the cortical cells increases slightly as the hydrolysis of the starch continues, but most of the drops remain very small except in the peripheral cells below the exodermis. In these cells large oil drops were frequently seen.

Occasionally the oil in a cortical cell collects and forms a thin film on the outside of its protoplasmic membrane. When this occurs the cells are very conspicuous, for they retain their contents, as the oil film prevents further diffusion from the cell.

As the hydrolysis of the starch proceeds, oil accumulates in the cells of the stele, so that at certain stages a considerable quantity of oil is present and large drops are found in the cells of the phloem, of the associated parenchyma, and of the pericycle. The amount of oil becomes less when most of the starch has disappeared, and is practically absent when depletion is complete. It remains longest in the cells of the stele.

vi. *Other cell contents.* In many of the tubercles examined at the stage when the starch had disappeared, some of the cortical cells were seen to be filled with fine granules which made the cells appear grey. These granules were not found at a later stage. The composition of the granules was not determined; they are soluble in alcohol and give no decisive indication of their nature with any of the usual microscopic reagents.

During depletion strands of 'mucilage' were seen in the tubercles; these were noted as apparently going from cell to cell: they were seen in the intercellular spaces and also in some cases in the xylem vessels. Attempts were made to determine both their origin and their composition, but without success. Similar strands were also found in other parts of the plant.

While depletion of the storage cells has been going on the tubercle has developed into a small plant, such as is depicted in Fig. 5, D, utilizing in its growth the cell contents of the storage organ. The gradual disappearance of the cell constituents as development proceeds has just been described; but, in conclusion, one would like to emphasize the fact that the total content of the cell is utilized, the starch, the amyloplastids, the cytoplasm, the nucleus, all disappear. Even the greater part of the cell sap is absorbed, as is shown by the contraction and wrinkling of the exhausted tuberous root, for this wrinkling disappears if a piece of the epidermis is removed and water allowed to enter the tubercle. The cortical cells break down after the extraction of their contents and the tuberous root decays.

VII. SUMMARY.

1. Some plants of *Ranunculus Ficaria*, L., are reproduced vegetatively by means of little tuberous structures which arise in the axils of the foliage leaves. These structures were called 'tubercules' by the earlier writers, and the term is used in this paper.

2. A tubercule, as recorded by most of the earlier investigators, consists of the axillary bud of a leaf and its adventitious root or roots. The bud remains small; the adventitious root becomes tuberous, the reserve food material, starch, being stored in its cortex.

3. Additional details are given of the anatomical structure of these adventitious roots.

4. These roots are specialized storage organs and take little or no part in the absorption of water.

5. The growth of the tubercule during its first year is described.

6. The deposition of starch in, and its removal from, the tuberous root of the tubercule were followed. Sugar passes down the stele of the developing root and starch is deposited in the cortical cells of the root from the bud end downwards and from the stele outwards. The starch is removed gradually from the cortex and disappears first from the cells in its central region. The effect of this regional removal of the starch is very striking in the later stages of starch depletion; the probable causes of the resulting zonation are considered.

7. A demonstrable amount of diastase is present in the tubercule while the starch is being removed, and appears to be evenly distributed in the storage tissue.

8. The cytological changes that take place in the cortical cells during the extraction of their contents are described. These may be summed up as follows:

(a) The starch grains hydrolyse evenly over the surface, for they gradually become smaller. During hydrolysis the grains remain clustered round the nucleus.

(b) The amyloplastids persist in the large storage grains, since they are to be seen after the starch has disappeared.

(c) The form of the nucleus is not constant. It varies at different stages; at first spherical, it becomes 'lobed' when metabolism is active, then generally becomes spherical again before it finally disappears. This alteration in shape is thought to be due to the special metabolic conditions existing in the cell.

(d) The amount of cytoplasm present in the cell gradually decreases until none is left.

(e) Small drops of oil appear in the cortical cells as the starch is

hydrolysed, and large drops are found in the cells of the stele, more especially in the cells of the phloem.

(f) Granules of some substance are noted as occasionally found in some of the storage cells after the starch had disappeared; the nature of the substance was not determined.

(g) Strands of 'mucilage' are present in the tubercule as in other parts of the plant. These were not seen in the exhausted root.

9. Active circulation of the cytoplasm of the cortical cells was observed during depletion.

10. All the contents of the cells of the storage root of the tubercule are utilized during its growth.

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Studies in the Cytology of the Hibisceae.¹

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With Plates XXXI-XXXIII.

INTRODUCTION.

THE Malvaceae is an order of plants containing some of such economic importance as to make it occupy from the point of view of utility a position perhaps only second to that of the Gramineae, which is the great source of food supply to the human race.

The Cotton plant (*Gossypium*) and the Hemp (*Hibiscus cannabinus*) are of such value for textile and other purposes, that the attention paid, in the regions where they grow, to their production and improvement is also only second to that given to the food crops.

Both of the genera *Gossypium* and *Hibiscus* are embraced within the sub-order Hibisceae. They, with other genera of the Hibisceae, present to plant breeders who have studied them closely a phenomenon of interest. This phenomenon is that, whether the divergence between the species of the different genera be great or small, there is often to be found, in a group possessing common specific characters, a characteristically constant variation in certain characters of a less ordinal value than the specific features. Not only are these peculiar variations of some characters to be found in different species of a genus, but the same sets or groupings of variations occur in the different genera. It seems evident that there is the same fundamental reason behind these variations whatever it may be. For instance, the pair of characters, the presence or absence of a red base or 'eye' in the petals, is one of these variations. Its absence from the New World group of different types of *Gossypium* is as constant as is its presence in the types of *Gossypium* of the Old World. In the species of *Thespesia* the same pair of characters is found. Another characteristic variation, the wide or narrow lobes of the leaf, is found commonly in the Old World types of *Gossypium*,

¹ Thesis approved for the Degree of Doctor of Philosophy in the University of London.

but not in those of the New World. The same variation is found in *Hibiscus cannabinus* and in the many horticultural varieties of *Hibiscus rosa sinensis*.

The important economic characters of comparative length or shortness of the seed-coat hairs is another allelomorphic pair of variations found in *Gossypium* and *Thespesia*. An enormous amount of attention has been given to the investigation of the morphological and genetic aspects of this variation in *Gossypium*. The remarkable polymorphic hairs of *Thespesia* seem to have received no notice.

The presence of red colouring matter in the cell-sap (a separate character from the red 'eye' in the petals) is a variation in *Gossypium*, *Hibiscus cannabinus*, and other species of *Hibiscus*. Comparative hairiness of the stem or leaf is another common variation in *Gossypium* and many of the Hibisceae. All of these variations mentioned are constant characters, which when once originated, are passed on from generation to generation. In *Gossypium* certain of them are known to have originated suddenly in the offspring of individuals which did not themselves previously possess them.

An extensive study of the Old World group of *Gossypium* varieties in the field suggested mutation phenomena comparable with those of the classical *Oenothera* of de Vries (14) as being possibly an explanation of the many varieties.

It was considered that an exploration of the cytology of the Hibisceae, as Gates (7) had done for the *Oenotheras*, would probably give some interesting results and throw a light upon the problem.

It was somewhat strange that although plant breeders should be giving so much attention to some of the Hibisceae, yet the cytology of the group, which might serve as a means of assistance, had been but little investigated.

Except for the scant accounts of Cannon (1) and Balls (8) of *Gossypium*, practically nothing was known of nuclear behaviour amongst the Malvaceae until 1924, when Denham (4) published an account of 'Microspore Formation in Sea Island Cotton', and 'Chromosome Numbers of Old and New World Cottons'.

The observations of Denham did not reveal any suggestions of nuclear behaviour that might indicate mutation phenomena within the genus *Gossypium*. They did, however, show that one group of cottons had arisen from the other by duplication of the chromosome numbers.

This was an important advance in our knowledge, and it was thought that a general occurrence of similar phenomena might perhaps be found in the Hibisceae.

The nucleus of the Hibisceae generally presents great difficulties in its study. Firstly, there is the comparatively small size of the nuclear struc-

tures; secondly, the number of the chromosomes is generally a high one, and such as to make it impossible to follow them individually at different stages.

An attempt was made to find forms with the nuclear structures of as large a size as possible, and with the chromosomes few in number.

The developing microspore was in all cases the material upon which the observations were made. Thirteen species of the genus *Hibiscus* alone were investigated, the anthers in various stages of development being sectioned and microscopically examined to find chromosome plates, so as to determine the suitability of the type for further study. Of all these, *Hibiscus tricuspidis* showed chromosomes of the largest size. The number of its chromosomes, as seen in the equatorial plate of heterotypic division, was forty. *Hibiscus tiliaceus* showed forty-eight smaller chromosomes in the heterotypic plate. Usually the chromosomes were too numerous to count with any degree of accuracy. *Hibiscus rosa sinensis*, for instance, in several counts of different plates showed seventy-two chromosomes upon its heterotypic plate. The numbers were, however, too high to enable one to give them with absolute certainty, or to follow the process of nuclear division (Pl. XXXI, Figs. 1, 2, 3).

On attempting to investigate the cytology of microspore formation in *Hibiscus tiliaceus*, another, and previously unforeseen, difficulty presented itself. This was the discovery of the fact that there was some discrepancy between the number of the chromosomes at heterotypic diakinesis and the number that were upon the spindle just prior to anaphase. The number in the latter case was certainly less than the former, and some of the chromosomes upon the spindle were seen to be larger than others (Pl. XXXI, Fig. 5). A similar discrepancy between the numbers at diakinesis and upon the equator of the spindle was noticed in *Gossypium barbadense* (Pl. XXXI, Fig. 6). Whether the reason for this difference in number of the chromosome masses is of the same nature in all cases cannot at present be said. From the observations that have been made, there is an indication that in the genus *Hibiscus* the chromosomes are a simple multiplication of four as a factor, or more probably still, in the light of information gathered from the study of *Thespesia*, eight. The anomalous numbers of thirteen for *Thespesia* and *Gossypium* are perhaps explicable derivatives of the number eight.¹

The number of the chromosomes in *Hibiscus tiliaceus* proved to be too many to enable one to follow their behaviour individually. No type had so far been found with chromosomes even as large as those of *Gossypium*, and

¹ Since the above observations were made, further investigation, described later in this paper, has revealed the probability of the factorial number in *Thespesia* being 6 or 7 rather than 8. This would perhaps indicate an error in some of the above counts. Unfortunately, in spite of several attempts, it has not so far been found possible to count the chromosomes in the somatic cells, which would afford a check in the matter.

the small size of the chromosomes in *Gossypium* had always proved an obstacle to previous workers.

At this stage, *Thespesia*, a plant with close relationship to *Gossypium*, was fortunately examined. Its nuclear structures, although small in comparison with those of many plants, are larger in size than any so far found in the Hibisceae. Of great assistance in its investigations has been the fact that its bivalent chromosomes at certain stages are of a characteristic cross shape, thus enabling them to be distinguished from univalent forms. Generally in the Hibisceae the chromosomes do not show this form. In number the chromosomes of *Thespesia* and *Gossypium* are similar, as already mentioned.

OBSERVATIONS ON THE CYTOLOGY OF MICROSPORE FORMATION IN *THESPESIA POPULNEA*.

Of the four known species of *Thespesia*, two are found in India, where the material for the following observations was obtained. *Thespesia populnea* is a small tree attaining to a height of some 20 to 25 ft. The other Indian species, *Thespesia macrophylla*, is an under-shrub of from 3 to 4 ft. high. They are typical Malvaceous plants with morphological characters suggesting nearer relationship to *Gossypium* than to other genera.

All the plants of the Natural Order Malvaceae contain mucilage within their tissues, and this secretion is especially copious in *Thespesia*. The abundant presence of this substance presents some difficulty in the preservation of material for microscopic observation. Not only does it prevent or impede the penetration of fixative solutions to the cells, but its affinity for water and swelling propensity in that fluid cause so many of the cells whose behaviour it is desired to see to come off the glass slip during the process of manipulation. Of many different fixative solutions tried, the stronger Flemming's solution, followed by a longer immersion in weak chromic acid solution, gave the most satisfactory results. Carnoy's fluid overcame the difficulty of inability to immediately soak the anther due to the presence of wax, but on the whole did not give so good preparations as the Flemming's solution did.

A modification of Carnoy's formula, made up so as to contain 3 per cent. of glacial acetic acid, however, proved to be of very great value in the work. This fluid causes the chromosomes to swell, and thus often makes the distinction between univalent and bivalent chromosomes plainer.

Generally, observations have been checked on material preserved by different fixing solutions. For staining the microscopic sections, safranin and gentian-violet solutions have been largely used. Heidenhain's iron-haematoxylin, followed by orange G, has also been employed.

The following observations refer to *Thespesia populnea* :

The anther sacs are curved back on each side of the filament into a U-shaped body with one limb of the U longer than the other (Pl. XXXI, Fig. 7).

Within the developing anther the sporogenous tissue forms a plate only one cell thick, except, that at first during rapid growth, cells may be squeezed out of this plate to lie above others, or wedge in wherever they can find a space (Pl. XXXI, Fig. 8). As the cells of the sporogenous tissue increase in number, a very delicate wall is formed between them. This cell membrane is later increased in thickness by a secretion on its inner side of a secondary substance of a clear and gelatinous nature, the so-called hyaline zone. The degree of deposition of this hyaline zone may be made to serve at times as a help in deciding the sequence of events in the life of the cell about to be described.

The nucleus of the cells of the generation, previous to that in which meiotic division occurs, shows the presence of much chromatic material in what might be termed deposits rather than granules (Pl. XXXI, Fig. 9). These are to be found situated upon a fine thread. More definite chromatic globules are also present in considerable number. The centre of the nucleus is occupied by a large nucleolus. The entire nucleus is surrounded by a delicate pellicle, the nuclear membrane.

The nucleus of the spore mother-cell shows a single spireme thread with embedded granules (Pl. XXXI, Fig. 10). Other and larger granules are usually present in the nucleoplasm, and these are generally situated at the points where the spireme threads cross one another. A characteristic nucleolus is also seen. In this condition the nucleus is more or less centrally situated within its cell. The spireme thread at this stage is loosely interwoven throughout the nuclear cavity. Profound physiological activity is obviously taking place within the nucleus. The spireme thread has become much more clearly defined, and the irregular deposits seen in the nucleus of the previous generation have been absorbed or dispersed in some way. The nucleus itself has increased largely in size.

There follows a stage when the nucleus usually migrates more to the surface of the cell-mass. This is accompanied by a close tangling up of the nuclear thread, which falls to one side of the nuclear cavity, the rest of the nucleus being more or less clear (Pl. XXXI, Fig. 11). This is the synizesis phase. The nucleolus can be seen sometimes during synizesis covered up within the tangled thread. It would seem to persist throughout this stage. No nuclear membrane can now be made out. Owing to the tight balling up of the contents of the nuclear cavity, it is not possible to see what actually happens during synizesis. Sometimes a loop of the spireme projects from the general mass.

The spireme thread when it passes into this synizesis stage is thin.

The thread now unravels from its tangled mass to again fill the cavity of

the nucleus (Pl. XXXI, Fig. 12). The nucleolus is still present. The thread can now be seen in many places to be double, consisting of two distinct rows of granules, each row embedded in a line of substance, and the two rows situated very close and parallel to one another. In many cases, certainly, the granules on one line are situated directly opposite the granules on its parallel neighbour (Pl. XXXI, Fig. 13).

The thread next thickens further, apparently this time by a process of contraction, for it presents the appearance of having less length than previously (Pl. XXXI, Fig. 14). Granules can be distinctly seen embedded within it along its length, but they no longer show any duplicate side-by-side arrangement. The condensed or thickened thread now throws itself into loops. Typically the loops are at first like U's. They all come out from a ball-like tangled mass of thread, in which their two limbs are buried, so that they appear as inverted U-like outgrowths. Some of the loops grow long in comparison with the distance between their parallel limbs; others do not, being more circular in form in consequence. The tangled mass from which these loops project is situated towards one pole of the nucleus, and near by the nucleolus is also situated. The longer loops extend just within the periphery of the nuclear cavity to near the opposite pole of the nucleus. The shorter and rounder ones make but little outgrowth from the tangled mass from which they originate. Viewed from the pole towards which is the origin of these loops they would seem to be some eight in number (Pl. XXXI, Fig. 15).

It is not possible to be certain of absolute accuracy in counting them, but their number can be stated safely to be nearer eight than thirteen. The reason for this observation will be apparent later.

The cavity of the nucleus away from the tangled mass will, at the above stage, be free from the spireme thread. Indeed, it is possible to get a diametric section at this stage showing little more than an empty circle. This is the stage often spoken of as that of 'second contraction'. The chromosome lengths of the spireme have evidently conjugated by the telosynaptic method of Farmer (10 and 11).

Next the loops twist upon themselves (Pl. XXXI, Figs. 16 and 17). The beginning of this twist is often to be seen as a single crossing of the ends of the two limbs of a loop. Eventually, however, the twisting becomes much tighter and extends throughout the entire length of the two limbs (Pl. XXXI, Fig. 18). The twisting of the loops upon each other continues until a very closely convoluted rod results, and ultimately to such an extent that the convolutions seem to blend to form a uniform rod. When the final stage has been reached no signs of the original two limbs can be recognized, and the rod shows no appearance of being double. The rod is now a short thick structure, often appearing as if with four granules embedded along its course. It is not possible to decide whether this appearance be actually due to

granules or to the optical effect produced by the two original threads crossing one another at these four points.

These rods that result from the twisted loops obviously, from their method of origin, represent bivalent prochromosomes (Pl. XXXI, Fig. 19, *a, b, c, d*). Usually any nucleus is not found in a uniform stage of development. There may be some loops, some rod-shaped pieces, and some of the earlier formed rods may be preparing for the next phase. For this reason, and also because there is a tendency to skip or abbreviate some of the intermediate stages, unless carefully looked for and suitable methods of staining employed, the following stage especially may be missed. The double staining of the preparation with safranin followed by gentian violet has given the most satisfactory results. The rod-like pieces may or may not have broken away from the point of their common origin by this time.

These rods next untwist so that they split or separate longitudinally into two threads again. The two longitudinal threads or halves usually, though not always, remain fastened for some time to each other at their ends, and they cross over at their centre, as if one more half-turn were required to completely untwist them. Their shape will thus now be that of a figure of 8.

(It is interesting to note that these figure-of-8-shaped chromosome loops had been seen and figured in the Malvaceae, in the case of *Gossypium*, by Cannon (1), before Janssens (9) used them to develop his theory of chiasmatypy. There are not many records of them in the flowering plants. Gates (2) reports them in *Lactuca*. They can also be seen in *Bombax malabarica* and *Hibiscus tiliaceus* amongst the Malvaceae.)

A granule of chromatic substance appears situated at each extremity of the figure of 8 in which the ends of the two longitudinal halves join. Sometimes a pair of little horn-like projections can be seen sticking out from this granule (Pl. XXXI, Fig. 20, *k*).

Usually the thread forming this figure is uniform in thickness throughout its length, with a few granules embedded in it. The granules on the two threads sometimes show a similarity in position. At times the thread of this figure of 8 is drawn out in places into a thinner portion, when an attenuated figure results.

The extremities of the two threads next separate at either end, each extremity retaining a moiety of the granule in which they were previously embedded (Pl. XXXI, Fig. 20, *f* and *h*).

The appearance of the bodies will now be that of an X-shaped figure.

The point where the two portions touch or cross over, however, at this stage seems often more towards one end than to the other, giving the appearance of a pair of pincers with jaws shorter than the handles.

It is to be noted that loops are frequently found with more than one cross over in the middle (Pl. XXXI, Fig. 20, *k*).

The limbs of the X-shaped structures increase in thickness and become shorter (Pl. XXXI, Fig. 21, *l*). The extremities of these opposite-angled limbs may now show the granule more pronounced, it often appearing quite globular. These extremities next curve inwards, the two of the limbs of each side curving in towards the angle they contain (Pl. XXXI, Fig. 21, *n*). Sometimes one side of the cross will be seen to have completed the above process before the other has started (Pl. XXXI, Fig. 21, *m*).

There is no doubt that at the above stage the two portions do not actually cross over one another. At the figure-of-8 stage they did. They are now to be regarded more as a very narrow-waisted H, or X, in which the two angles only touch.

Observations leading to this interpretation are supported by such cases as where one side develops before the other. The change from a crossed to a non-crossed stage is only a matter of altering the planes in which the limbs lie, as is easily seen on performing the experiment with two lengths of some plastic substance or string.

The two extremities of each side having met in the angle, a globular body results, two of which, when both sides complete the operation, would be situated side by side. Each will have been formed from what were the arms or limbs of the right angle. They may at first show a constriction where the original angle of the limbs was as though divided at the centre (Pl. XXXI, Fig. 21, *m*); but this disappears and the whole becomes globular. The product of each side obviously is a univalent chromosome.

There seems to be a great tendency to curtail the history of development as we have just stated it. This results in many irregularities and departures from the course given.

Frequently a trident-like body can be found with three limbs coming off from the fourth. This is obviously only the limbs of the original cross somewhat displaced.

At times, instead of the four-limbed cross X, a three-limbed Y is seen, two limbs presumably remaining unseparated (Pl. XXXI, Fig. 21, *o*).

A comparatively thick semicircular rod is often seen with its ends each terminating in two fused granules (Pl. XXXI, Fig. 21, *p*). This is evidently the two right angles of the cross apposed and their contained angle rounded out.

The chromosomes, as described above, have never been counted at this stage in full complement in any one cell. They do not develop at all contemporaneously and it is not possible to count them up to this stage.

Whether or no all the cross-shaped bodies form a chromosome from each half cannot be said. It has already been mentioned that one half has been seen to develop before the other has started.

No diakinesis stage with the chromosomes showing a definite or recognizable bivalent form has been seen in the prophase of heterotypic division. Gates (3) comments upon the same fact in *Oenothera*. When the full com-

plement of chromosomes has been first seen within the nuclear cavity they have then all assumed a more or less globular form (Pl. XXXI, Fig. 22).

The nucleolus at this stage is no longer to be seen. As the prophase has proceeded an area around the nucleus has become sharply marked off from the rest of the cell cytoplasm. It originates at about the time of the completion of the formation of the rods from the spireme. Up to the strepsinema stage, when the loops twist together, there is no evidence of its existence. It begins immediately next the nuclear cavity by the protoplasm there becoming darker in appearance. From this stage it increases, attaining its maximum thickness when the nucleus has reached the metaphase. This is the so-called perinuclear zone. The perinuclear zone is well marked in *Thespesia*, but its structure is not well shown at this stage as its appearance is more granular and pigmented than in some forms.

By the time that the chromosomes are fully formed within the nuclear cavity, this space is found to be pervaded by a mass of fibres whose ends seem ultimately to concentrate at three or four points on the periphery of the nucleus. This multipolar spindle next becomes a bipolar one by the several poles of the previous multipolar arrangement concentrating at one or other of two opposite points. The cell itself has passed from a spherical to an ovoid form.

The chromosomes are now arranged in a scattered plate upon the equatorial plane of the spindle. Their number is thirteen. They have been frequently counted in different cells (Pl. XXXI, Fig. 4).

Most of them are circular in outline at this stage; a few may be oval or kidney-shaped. The chromosomes next pack together at the centre of the nucleus. Eventually they form here such a compact mass that often individual chromosomes cannot be recognized. At their first accumulation at the centre many or all of the chromosomes can be seen to have lost their globular form and to have become somewhat vermiform in outline (Pl. XXXI, Fig. 23). Sometimes the chromosomes at this stage are of a tadpole-like shape with a globular enlargement at one end and tapering to a point at the other. The chromosomes now certainly resemble the univalent halves of the X-shaped bodies previously described. The tadpole-like form can be accounted for on the assumption that one arm enclosing an angle has not uncurved, whilst the other has.

This massing at the centre of the nucleus is believed to be a natural occurrence and not due to faulty fixation of the preparations in which it has been observed. The preparations otherwise show the fixation to have been good. Also, sometimes two or three chromosomes have not joined the main mass, which they might have been expected to have done, if faulty fixation were its cause.

A massing of the chromosomes at the centre of the nucleus, in the case of *Gossypium*, has been described and figured by Denham (4). He evi-

dently thinks, however, that in *Gossypium* this massing occurs before the arrangement in an equatorial plate. In *Thespesia* it certainly occurs after that stage. The sequence of events in two such stages may not always be easy to decide.

Denham states: 'It is believed that the migration of the chromosome masses from the periphery to the centre of the nucleus is brought about by the contraction of the connecting fibrils with the simultaneous elongation of the radiating or spindle fibrils (Pl. VIII, Fig. 15). (Traces of the connecting fibrils can still be seen in the diaster as a fine reticulum uniting the chromosomes.) The whole body of the chromosomes in diakinesis passes evenly and simultaneously to the centre of the nucleus, where at first they form a dense and almost spherical aggregation (Pl. IV, Fig. 34; Pl. VIII, Figs. 16, 17), though an occasional laggard chromosome may follow at some distance. . . .' This description applies exactly to what has been observed in *Thespesia*, provided that we interpolate the arrangement of the chromosomes on the equatorial plate before they form an aggregation at the centre of the nucleus.

Davis (5), in the case of *Oenothera grandiflora*, describes the chromosomes at the centre of the nucleus as lying 'in a group, so closely massed that their arrangement can be made out only with difficulty'.

In *Thespesia* the chromosomes emerge from the central mass as eight bodies and not thirteen as they went in (Pl. XXXII, Fig. 25).

We shall leave any possible explanation or discussion of this reduction in numbers until we have completed the account of the observed facts up to the time of formation of the pollen grain.

The eight chromosome masses arrange themselves as an equatorial belt upon the achromatic spindle (Pl. XXXII, Figs. 26 and 27). They are generally regularly arranged, and when seen in longitudinal view four only are in focus at one time, but the other four can be found by changing focus.

Often the masses on the equator are in contact, touching one another, but sometimes there is a space between them, and then line-like connexions have been seen between neighbours.

When first assembled on the equator the eight masses appear fairly uniform in size and of a globular shape.

This is the completion of the metaphase.

HETEROTYPE ANAPHASE.

The following anaphase is evidently one of but short duration, for it is the least common stage found in preparations. Its initiation is evidenced by the chromosome masses being drawn out into angles, and sometimes they can be seen as if separating into two.

There is a great deal of variation in the stage of migration of the chromatic material from the equator to the poles of the spindle.

We will describe all the types that have been observed.

1. The chromosome masses on the equator divide so as to give five bodies which travel towards one pole and eight towards the other. Some of the masses lag behind the others. Some leave a long trail of chromatic material behind them, which often connects with a chromosome mass going to the opposite pole. The masses are sometimes drawn out into attenuated shapes (Pl. XXXII, Fig. 28). The whole arrangement is most irregular.

Three of the chromosome masses, which will henceforth be referred to as the 'peculiar' chromosome masses or chromosomes, do not always move with the rest. At times they seem to move later than the others and can be found on the spindle when the others have already reached the poles (Pl. XXXII, Fig. 29). At other times they appear as if they had travelled first and lie almost beyond the spindle apex where the others are (Pl. XXXII, Fig. 30).

It might have been expected, perhaps, that a type of heterotypic anaphase would occur in which thirteen bodies went to one pole and ten to another. That form has not been found. It does seem, however, that sometimes division does occur so as to give more than five masses to one pole and eight to the other. Evidence of this is given in the case of sister nuclei which later show retarded homoeotypic division owing to the presence of more than three univalent-like chromosomes within them. These cases will be mentioned later.

2. A second type of anaphase at heterotypic division occurs.

Characteristic of this type of anaphase is the fact that the chromosome masses move apart from the equator in a plate-like group (Pl. XXXII, Fig. 31). It is not possible to distinguish individuals in this mass. (The possibility of this appearance being the result of treatment in the preparation of the material must not be overlooked.)

3. A third type of anaphase has been rarely found. In this three chromosome masses have been seen going towards one pole and five towards the other.

TELOPHASE AND INTERKINESIS.

There are two distinct types of conditions found at early telophase. Whether one belongs to the first-mentioned form of anaphase and the other to the second could not definitely be said without observation upon the process of division in the living cell.

It is not known if the third type of anaphase ever advances so far as the telophase stage.

Often on reaching the poles of the spindle the chromosomes are aggregated into a dense nodular mass in which at first no individuals can be

seen (Pl. XXXII, Fig. 29). Later this mass opens out somewhat, and separate bodies can be distinguished. This fused mass at the commencement of telophase is a condition described by Lawson (6) in *Passiflora caerulea*.

It does not appear always to occur in *Thespesia*, but whether it be characteristic after one type of anaphase more than after another cannot be said.

At other times, just prior to reaching the summit of the spindle, ten club-shaped bodies with their swollen ends hanging towards the equatorial plane have been seen (Pl. XXXII, Fig. 32). These are apparently the clubbed ends of five U- or V-shaped bodies.

In another preparation (Pl. XXXII, Fig. 33) ten bodies are seen viewed from the equatorial plane. Around the chromosomes in this specimen is a ring of finely granular substance from which radiate fine lines of a similar nature. The bodies themselves are situated within a vacuole-like space which the granular ring bounds. The granular ring is the beginning of a new perinuclear zone.

In another ventral view the three peculiarly behaving chromosomes have been seen lying to one side of a somewhat indefinite nodular mass (Pl. XXXII, Fig. 34).¹

Having reached the poles of the spindle the chromosomes lie within a vacuole which increases in size, whilst, at the same time, the chromosomes themselves get farther and farther apart.

Around this vacuole is the perinuclear zone. Between the two

¹ The method and results of heterotypic division as observed at the opposite poles of the spindle were for some time very bewildering. Two types of phenomena at telophase may be seen; there may be five bodies at one pole and eight at the other (Pl. XXXII, Fig. 30), or there may be ten bodies at one pole and thirteen at the other (Pl. XXXII, Fig. 35). To understand these different conditions it is best to visualize matters by making models. Two X-shaped cross-like pieces may be cut out of stiff white paper; or, to make a model more realistic, two figures of 8 may be cut out, their upper and lower extremities being cut apart, when a cross will result. These crosses consist of two lines intersecting. In one cross a line may be coloured, say red, and represented as if lying on the top of the other (the white) line at the crossing point. In the second cross the similar line of the pair may be coloured red. These models now represent the X-shaped chromosomes that were seen at prophase. With each cross the process can be imitated by which the crosses were seen to form globules. Fold a red arm (that is, half a red line) over upwards so that it comes to lie in contact with its other red arm, and fold a white arm downwards so that it lies in contact with its other arm. At late prophase ten bodies fuse in pairs so that from the thirteen bodies seen at diakinesis eight masses form and assemble upon the equator of the spindle. To imitate this fusion put the two pieces, *folded up* as just described, one on top of the other so that the red part lies on the red and the white on the white. To realize the division that follows at anaphase, imagine the two as last placed torn or pulled apart so as to separate all the red from all the white. We now have a red and a white cross (folded up it is true) that travel each to opposite poles of the spindle. This is a qualitative separation. With five of these to one pole go the three 'peculiar' chromosomes. This gives the condition of five and eight bodies at opposite poles at telophase. As will be understood from the model five bodies at each pole are composed each of the four limbs of a cross. Even when globular or bulbous in form this compound nature can often be seen in them as dot or line edges to them (Pl. XXXII, Fig. 36). As the two halves of each of the five crosses (folded up or condensed into a globular form) at the spindle poles are derived one from each of two different original crosses that fused, they may transiently show separately as halves (globular in form), thus giving ten (2×5) and thirteen ($2 \times 5 + 3$) bodies at opposite poles (Pl. XXXII, Fig. 35, and also no doubt this is the explanation of Pl. XXXII, Figs. 32 and 33).

perinuclear zones, one at each pole, the former spindle fibres at first stretch.

The spindle fibres are replaced shortly by radiating outgrowths from the developing two new perinuclear zones. These radiating outgrowths can be recognized coming off from the perinuclear zone as soon as it begins to form around each daughter nucleus at the poles of the spindle (Pl. XXXII, Fig. 33).

The formation of the perinuclear zone and its radiations is best seen at this stage. It is to be remembered that one already had formed around the single nucleus at the initiation of the heterotypic division. This zone is only found at the time of heterotypic and homoeotypic division.

The perinuclear zone is formed around the nucleus as soon as the vacuole of karyoplasm begins to be secreted around the chromosomes. It separates this vacuole from the general cell cytoplasm into which it and its radiations eventually extend.

The nuclear membrane is afterwards formed on the inside of the perinuclear zone.

By the secretion of more and more karyoplasm the nucleus enlarges and the chromosomes are separated more and more from one another. An interkinesis stage next follows, during which the chromosomes disorganize and then re-form, their identity, for a time, being lost. This interkinesis stage is, however, obviously a variable one. In some nuclei it is prolonged and complete, disorganization and reconstruction of the chromosomes being along regular stages; in other nuclei the chromosomes perhaps pass to homoeotypic division without losing their identity.

In prolonged interkinesis the chromosome material breaks up into small fluid-like globules which become scattered throughout the nuclear cavity (Pl. XXXII, Fig. 37).

The nucleus now contains one or more small nucleoli.

When stained by long immersion in safranin solution a delicate network can be seen to traverse the nucleus. The globules are situated along the fibrils of this network.

In some nuclei two distinct sets of network are to be seen (Pl. XXXII, Fig. 38). The assumption is that these are the daughter nuclei into which the three peculiar chromosomes went and that their material keeps separate upon a network of its own.

After a period of rest the chromosomes reconstruct. At points along the nuclear thread or network a globule, usually larger than the others near it, may be seen from which the thread forks in front and behind, giving a spider-like appearance (Pl. XXXII, Fig. 39). Smaller globules are situated along the leg-like threads. This is obviously the reoccurrence of the stage in the formation of the cross-like chromosomes seen before in the prophase of the heterotypic division.

Some chromosomes can be now recognized in the nucleus as very definite cross-shaped bodies. Usually one hugs the nucleolus (Pl. XXXII, Fig. 40).

The three peculiar chromosomes reappear at interkinesis. They never show as cross-shaped bodies however, but as single, often bent, rods.

The disappearance and reappearance of the chromosomes during interkinesis is along the lines described by Digby (12) in *Osmunda*.

Counts of the chromosomes in the nuclei at this phase have been made. The method found satisfactory is, firstly, to use material that has been fixed in the modified Carnoy's fluid with the extra acetic acid, as described previously. This causes a swelling of the chromosomes. An eyepiece with crossed rulings, dividing the circle into four quarters, is used on the microscope, and the centre of the nucleus is so placed on the stage that the intersection of the crossed lines coincides with it. By concentrating on one quarter of the nucleus at a time and focusing up and down all the forms seen can be plotted within a circle divided into quarters on a sheet of paper.

Five cross-shaped chromosomes in each nucleus with an extra three rod-like forms in one is a common case. Irregularities, however, are frequent.

At late prophase the chromosomes appear in sister nuclei as ten and thirteen globular bodies respectively, the cross-like forms evidently having divided into two, a right and a left portion.

HOMOEOTYPIC DIVISION.

The chromosomes come at late prophase on to the equatorial plate of an internuclear multipolar spindle. They are at first rounded or globular. The ten chromosomes in one nucleus are usually slightly in advance in development on the thirteen in its sister nucleus. The ten will often show a distinct split ready for division, whilst the thirteen will not have started to split. This is well seen in a fortunate section that may happen to pass transversely through two sister spindles that lie parallel to one another, or that may cut them obliquely so as to show the equatorial plates of both (Pl. XXXII, Figs. 42 and 43). Frequently, if not usually, the sister nuclei have their spindles so orientated that their long axes are at right angles to one another. It is interesting to note at this stage that in the little cytological work that has been done amongst the Malvaceae, working with New World types of *Gossypium* which he showed to be diploid forms of the Asiatic, Denham (4) disputes with Balls (8) because the latter only found twenty chromosomes whilst the former stated the number to be twenty-six—a dispute which recalls the classical case of Boveri and van Beneden over the difference of chromosome numbers in *Ascaris*.

There does not appear to be any massing of the chromosomes at the

centre of the cell prior to their arrangement upon the homoeotypic division spindle as there was at the previous division. No evidence of such has been found.

Ordinarily the chromosomes assemble on the equatorial plate as globular bodies. That they often fail to do this will be seen presently to be the cause of complications.

The thirteen chromosomes still frequently, if not always, form eight groups on the equator (Pl. XXXIII, Fig. 46). The nucleus with ten chromosomes does not show eight groups.¹ A type of anaphase with the chromosome material moving apart as two plates of fused masses is found at homoeotypic division just as it was at heterotypic. Whether this type completes its life-history as pollen grains is not known. There is no evidence to show that the three peculiar chromosomes divide at this stage. They have been seen at a pole of a homoeotypic spindle attenuated but obviously undivided (Pl. XXXIII, Fig. 49).

It is quite usual for one nucleus to have started on the anaphase stage before the chromosomes of the other have split, lost their cross-shaped form, and assembled on the equatorial plane. Probably they then never do lose the cross-shaped form and develop farther. As a rule, the nucleus that delays its development is the one in which the three peculiar chromosomes are to be found (Pl. XXXIII, Fig. 52). Sometimes this nucleus can be found to have abnormally increased in area as if it were growing in size, whilst its sister nucleus was dividing. The dividing or divided sister nucleus in this case atrophies first. There seems to be considerable mortality amongst the cells at homoeotypic division.

When the chromosome masses reach the poles of the homoeotypic division spindles they there form close aggregations, as were seen at hetero-

¹ At homoeotypic division it seems that the separation at anaphase is such that a univalent half derived from one original figure of 8 goes to one pole, and the similar univalent half of the other figure of 8 goes to the other pole. Evidence for this statement is furnished by such a case as that shown in Pl. XXXIII, Fig. 47, where a univalent chromosome is seen separating into four globular portions, the two going to either pole being themselves moieties (formed as indicated in Pl. XXXI, Fig. 21, *m*) of half of an original univalent length of the spireme, the half-length being the result of fragmentation. The double dumb-bell-like character of other chromosomes in Fig. 47 indicates the same thing. This is truly quantitative division, but differs from the ordinary conception of such division in not being a longitudinal splitting of the chromosome. It must be remembered that the meaning of the longitudinal splitting of the chromosome is to attain its separation into two similar halves, the word 'similar' implying sameness with regard to hereditary value. It will be pointed out later that there is a very strong suggestion in the chromosomes of *Thurberesia* that this can be attained by transverse fission. Of the eight masses upon the homoeotypic division spindle the five that divide may first do so into two bodies each, or they may straight away divide into four each, half the number resulting from the division in any case going to one pole, and the other half to the other (Pl. XXXIII, Fig. 47). The nucleus with ten chromosomes, as has been seen (Pl. XXXII, Fig. 42), even starts their division when on the equatorial plate. No nucleus has been found with ten chromosomes (nor with five masses) upon the equatorial belt at homoeotypic metaphase; the chromosomes have started to divide before they reach the equator. Very regular anaphase stages have been found, however (Pl. XXXIII, Fig. 48), showing ten chromosomes going to each pole.

typic division. Examples have not been found with the chromosomes arriving free at the spindle poles. Later the chromosomes become free as the karyolymph is secreted around them. If the chromosomes be found in a free stage in a nucleus they can sometimes be counted (Pl. XXXIII, Figs. 50 and 51). It is most unlikely, however, that more than one nucleus out of the four will permit of this.

One nucleus is usually a little, but recognizably, larger than the others. Sometimes, instead of forming a tetrahedron the four cells with their nuclei form a flat plate. In such forms one only of the four nuclei at interkinesis shows two sets of nuclear networks. These observations prove that three nuclei are indeed different from the fourth.

Telophase is followed by reorganization, during which the chromosomes appear in a very definite form. They show as three or four chromatic granules at the end of a delicate stalk-like thread. The chromatic granule at the head is the largest, and the others get successively smaller as they are situated more distally from it (Pl. XXXIII, Fig. 53).

In a very clear preparation it is possible at times to count the chromosomes in this form, but they are regularly distributed on the periphery of the nucleus and there may be difficulty, especially with those at the circumference of the circle. Later, this form of chromosome is not seen; in the nucleus of the pollen grain it is replaced by chromatic granules.

Each nucleus is surrounded by a very definite perinuclear zone, and all the perinuclear zones are connected with one another by radiating strands.

The tetrads are still enclosed, each within the thin walls of the original cell from which they have developed. Around the tetrad, within these walls, is a mucilaginous pellicle, the so-called hyaline zone (Pl. XXXIII, Fig. 54). This is evidently a secretion from the developing tetrad, but its deposition commenced even prior to heterotypic division. The four cells of the tetrad loosen or separate from one another internally. Externally, later they appear held together by nothing more than the radiations of the perinuclear zone. The mucilaginous material is now secreted in the hollow internal to the four cell masses and its deposition extends outwards so as to form plates between the cells. These plates ultimately connect with the outer sphere of hyaline material so as to divide it into four compartments, each containing a young pollen grain (Pl. XXXIII, Fig. 55).

SUMMARY.

The process of microspore formation in *Thespesia populnea* is described and figured.

1. Chromosome conjugation in *Thespesia populnea* is of the telosynaptic type.

2. Chiasmatic figures of the chromosome threads are present in the nucleus during heterotypic prophase.

3. During prophase development the chromosomes pass through a cross-shaped form, from which they emerge as globular bodies.

4. Thirteen globular bodies appear on the equatorial plane of the spindle, which then mass at the centre of the cell and fuse so as to form eight which regularly arrange themselves in a belt on the equator of the spindle.

5. Different types of anaphase occur.

6. A nodular mass is seen at the poles of the spindle at telophase from which the chromosomes separate out.

7. After a period of interkinesis the chromosomes are seen as cross-shaped bodies. There are typically five crosses in each nucleus and three extra non-crossed bodies in one of the nuclei. Variations of this arrangement however occur.

8. The chromosome bodies appear on the sister equatorial plates of homoeotypic division as ten and thirteen bodies respectively.

9. In the pollen tetrad three nuclei contain ten chromosomes and one thirteen.

DISCUSSION.

The question naturally arises—Can any explanation be given for the cytological phenomena as described in the foregoing account of microspore formation in *Thespesia*?

There is at first sight a superficial resemblance in the three peculiar chromosomes to the idiochromosomes in the nuclei of certain insects, as described by Wilson (13), but on deeper examination the idea does not seem to hold. The idiochromosomes of Wilson's types owe their peculiarity to deferred conjugation, and there is no evidence of this in the case of *Thespesia* so far as has been seen.

The possibility of *Thespesia* being a hybrid and the phenomenon in any way comparable to that found in cereals by Kihara, where there are peculiar chromosomes due to their non-pairing at fertilization, cannot be held.

The genetic behaviour of *Thespesia* has not been investigated, but its near ally *Gossypium*, in which there are the same chromosome numbers, has been subjected to intensive study by many workers. Such study has given no suggestion of a hybrid origin for *Gossypium*.

The peculiar phenomena found in the genus *Rosa* and described by Täckholm (16, 17), Blackburn and Harrison (18), and Hurst (19), were examined for a possible explanation. There is a similarity between *Rosa* and *Thespesia* in that bivalent and univalent chromosome forms appear upon the same spindle; also the mutation phenomena in *Rosa* resemble those in

the Hibisceae in being repetition phenomena in the different forms. The explanation by Hurst (19), however, that one species showed the combined characters of two or more species superimposed as it were one upon the other, disposed of the possibility, even in a simple form, of it offering any explanation for the mutation phenomena in the Hibisceae.

The mutation phenomena in the Hibisceae essentially consist in the alteration of a few characters in each form, and not the introduction of a number of new characters in addition to those already possessed. It may perhaps be contended here that too much consideration is being paid to genetic phenomena in this discussion. The whole purpose of the work is to find, if possible from cytological investigation, some facts that may aid in the understanding of the genetic behaviour in the Hibisceae. It seems, therefore, just as reasonable to use the effect to guide one to the cause as to use the latter to explain the former when it has been discovered.

It would seem that no previous observations in other plants can be used to explain the process in *Thespesia*. It is proposed, therefore, to enter somewhat into the realms of speculation, supporting it by observed cytological morphology where possible, to give a probable explanation of the phenomena.

Firstly, let it be remembered that the large numbers of the chromosomes and their minute size in many of the Hibisceae, which presented an impediment to the exploration of their cytology, are not incompatible with a suggestion of transverse fragmentation of the chromosomes having taken place, a possibility in the life of the chromosome admitted by Farmer and Digby (20).

In support of this suggestion, too, was the observation made at the time of second contraction in the heterotypic prophase that the number of the chromosome loops did not appear to be so many as thirteen.

The pincer-like form of the cross-shaped early chromosome also somewhat supports this theory. It represents a segment of what for convenience of description will be called an augmented figure of 8. Such shaped bivalent chromosomes were found at heterotypic prophase. The extreme circles of such a figure are usually more oval or elongated than the central ones; that accounts for the pincer-like forms with elongated handle-like limbs and shorter jaw-like ones (see Pl. XXXI, Fig. 20, *f*). They are formed by a fragmentation of the bivalent loops. A separation at their ends produces the cross-shaped form. If the paper models described (see foot-note, p. 766) be again used, the phenomena following the formation of these chromosomes can be better visualized. Stand the two crosses or figures of 8 in a line so that one is above or standing upon the other. This forms a model of the augmented figure of 8. Pick up one of the figures of 8: it represents a segment formed by transverse fission. It does not simply superimpose upon the other and then fold up into a globule in the manner they have been

found to do in the nucleus, and as is explained on p. 766, because in this condition the two univalent portions would be inextricably entwined with one another. This can be easily seen by performing the experiment with the models. If the two figures first be folded up separately in the manner previously described, and then be superimposed, the two univalents are in a condition so that they can slide apart. This explains what we actually see in prophase: the ten cross-shaped bodies first fold up into globules, with the three peculiar chromosomes they are seen upon the equatorial plane as thirteen bodies, ten of these bodies then fuse in pairs, the whole complement now appearing as eight.

The packing together of thirteen chromosome-like bodies at the centre of the cell at heterotype prophase and their appearance afterwards as eight in number is due to the recombination of ten half-chromosomes in pairs of halves. Boveri has made the comment on this phenomenon that when chromosomes fuse it implies that they carry similar characters—a comment that is very significant in the present case.

There is something suggestive in the account of mitosis in *Lepidosiren* as given by Agar (15), that there is there a phenomenon of chromosome segmentation and fusion similar to that in *Thespesia*. In *Primula kewensis* a fusion of bivalent chromosomes to form a quadrivalent one is reported by Digby (21).

So far no explanation of the three peculiar chromosomes and their consistently uncrossed form has been offered.

Within the nucleus, at the time that the figure-of-8 loops are formed, two bodies of a different and characteristic appearance have been found. They, and especially one of them which is the less likely to escape attention, have been seen in several nuclei. Constant similarity in size and form disposes of the possibility of these bodies being a chance occurrence. One of these bodies consists of two bowed or slightly curved threads with their concavities towards one another and crossing at their ends (Pl. XXXIII, Fig. 56). The other body, which is much the more difficult to find, consists of a straight thread. In one case (Pl. XXII, Fig. 56, *b*) it has a more delicate fibril hanging from it somewhat like the lash from a whip. These two types of bodies have been found in association in various nuclei, and it seems that there can be no doubt that they are definite developmental forms of some structures. The double bow-like figure has every appearance of being a figure-of-8 segment as it were, in which the univalent threads have not crossed over at the centre. Is the other body another segment in which one of the univalent threads has for some reason atrophied? A suggestion of possible atrophy in these threads has been seen before (see Pl. XXXI, Fig. 20, *g* and *j*). The opening of the double body at its ends, which on analogy with what we have seen to occur in the case of the figure-of-8 structure we should expect, would result in the production of two *univalent* chromosome threads from

it. If one thread atrophy in the case of another body only one univalent chromosome results and would explain the single thread form. This explanation would account for the production during the heterotype prophase of three chromosomes differing from the rest.

They differ from the rest in that their univalent or different qualitative portions have separated. That is what ordinarily takes place at the heterotype division. They may therefore be considered as having performed the equivalent of this division. We have seen that, at the homoeotype division which follows, the evidence (such as that afforded by Pl. XXXIII, Fig. 47) showed that the univalent bodies separated so that the portion originally contained in one figure of 8 divided from the similar portion contained in another. Now if the two bodies, the double bow-like one and the whip-like one, be moieties that segment off by fission as each a half of an original U-shaped spireme loop, and the other corresponding half of each loop does not segment off from the basal mass as seen in Pl. XXXI, Fig. 15, or for some reason atrophies, we should also already have had an equivalent separation to that which takes place at homoeotypic division. This would account for why these three peculiar chromosomes go to one pole without any corresponding bodies travelling to the other. The suggestion that the two bodies, the double bow-like one and the whip-like one, are each derived from separate spireme loops and are not sister fragments of one and the same seems supported by the fact that there is no attempt on the part of two of the three peculiar chromosomes to pair. This, if both were derived from one loop, might have been expected on the analogy of the behaviour of the figure-of-8 bodies.¹ So far as it can be ascertained, no case similar to that of these three peculiar chromosomes has been reported.

Wilson (13) has described in certain Hemiptera the phenomenon of chromosomes that show retarded conjugation. The present case would seem to be somewhat the converse of that, and be rather the precocious development of the chromosome at heterotype prophase up to a stage that would not ordinarily be reached until the completion of homoeotypic division.

The development of the megaspore of *Thespesia* and the behaviour of the three peculiar chromosomes at fertilization want exploration. The fact that there are two definite kinds of pollen grains regarded from the point of view of chromosome numbers presents in itself the possibility of mutation phenomena.

The corollary to this, that there is also likely to be further disturbance in the chromosome machinery at the time of microspore formation, introduces the possibility of still further mutation.

The genetics of *Thespesia* have never been studied; its nearest relative, *Gossypium*, has received continued genetic investigation. It has been

¹ If the peculiar chromosomes be derived from one and the same spireme loop, then the chromosome factor would be 6. If from two different ones, it would be 7. See foot-note on p. 757.

shown unknowingly that in *Gossypium* both of the forms of microspore perhaps do exist, and that *Gossypium* may have chromosome numbers like *Thespesia* (Balls (8) and Denham (4)). Within the genus *Gossypium* there are many varieties which will now no doubt be recognized as mutation forms. It has been demonstrated by Denham (4) to exhibit multiploidy in its chromosomes, a phenomenon often occurring in a genus showing other forms of chromosome variations. At least two historical and hitherto inexplicable cases are known of the sudden appearance of new types of cotton. One is that of the Sea Island cotton and the other of the modern Egyptian. It is possible that the study of microspore formation in *Thespesia* may now lead to an understanding of these and other forms. Further exploration may very likely give other possibilities of considerable economic importance.

I wish to acknowledge the kindly interest and encouragement that I have received from my one-time teacher, Professor V. H. Blackman. To the authorities of the Imperial College of Science and Technology my thanks are due for facilities afforded me, whilst on a period of leave from India, to complete my investigations in the Huxley Biological Laboratory. To Sir John Farmer I am especially indebted for the criticism and help that he has so willingly given me.

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EXPLANATION OF PLATES XXXI-XXXIII.

Illustrating Dr. W. Youngman's paper on Studies in the Cytology of the Hibisceae.

Unless otherwise stated, all the figures are drawn with a camera lucida at a uniform magnification of 1,140. This magnification was obtained by using a Leitz 1/16-inch immersion objective and a Leitz periplanatic eyepiece 10. A Leitz aplanatic condenser of N.A. 1.40 was used throughout. The condenser was immersed and Wratten colour screens were used, when necessary. Unless stated, all preparations were preserved in Flemming's fluid; those swollen with acetic acid are indicated.

PLATE XXXI.

- Fig. 1. *Hibiscus tricuspis*. The chromosomes on the equatorial plate at heterotype division. $\times 1,850$.
- Fig. 2. *Hibiscus tiliaceus*. As above. $\times 1,850$.
- Fig. 3. *Hibiscus rosa sinensis*. As above. $\times 1,850$.
- Fig. 4. *Thespesia populnea*. As above. $\times 1,850$. The chromosome masses do not show much inequality in size here, but see Fig. 22.
- Fig. 5. *Hibiscus tiliaceus*. A longitudinal section of the heterotype division spindle at metaphase. $\times 1,140$. Unequal chromosome masses are seen. The entire spindle occupies three consecutive sections, in each of which some chromosome masses are found.
- Fig. 6. *Gossypium barbadense*. Homoeotypic division spindle at metaphase. On altering the focus three more chromosome masses can be seen. The whole spindle is in one section.
- Fig. 7. *Thespesia populnea*. Curved anther sac with its filament between its limbs. $\times 20$ (about).
- Fig. 8. *Thespesia populnea*. Oblique transverse section of anther along the line indicated in Fig. 7. $\times 103$.
- Fig. 9. *Thespesia populnea*. Outline of a cell of the generation previous to that in which meiotic division occurs, showing the nucleus.
- Fig. 10. *Thespesia populnea*. Nucleus of the spore mother-cell.
- Fig. 11. *Thespesia populnea*. Nucleus of spore mother-cell, synizesis phase. The nucleolus is shown in circular outline.
- Fig. 12. *Thespesia populnea*. The nucleus with the thread unravelled from synizesis. Evidence of a double thread can be seen in places.
- Fig. 13. *Thespesia populnea*. A section through another nucleus in the same stage as the last. A very evident double thread is seen.
- Fig. 14. *Thespesia populnea*. The nuclear thread has thickened and become much more clearly defined.
- Fig. 15. *Thespesia populnea*. The nucleus, showing the origin of the spireme loops.
- Fig. 16. *Thespesia populnea*. The twisting of the spireme loops.

Fig. 17. *Thespesia populnea*. The extremities of the twisting loops.

Fig. 18. *Thespesia populnea*. The extremities of the loops. In two cases they are tightly twisted. The preparation has been swollen by treatment with acetic acid.

Fig. 19. *Thespesia populnea*. *a, b, c, and d*. Rod-like bodies formed from the tightly twisted loops.

Fig. 20. *Thespesia populnea*. *e, f, g, h, j, and k*. Loop-shaped bodies formed by the untwisting of the rod-like bodies.

Fig. 21. *Thespesia populnea*. *l, m, n, o, p*. Forms resulting from the loop-shaped bodies. These figures show well the method of formation of the globular bivalent chromosome. In *m* the right-hand side has almost rounded itself off into a univalent globule before the left-hand side has commenced to do so. In *n* the two sides are simultaneously forming each a univalent globule.

Fig. 22. *Thespesia populnea*. A cell showing the nucleus surrounded by the granular perinuclear zone within which thirteen chromosome bodies are seen. Three of these are somewhat smaller than the rest. One to the extreme right suggests by its shape that it consists of two fused globules formed as explained in Fig. 21.

Fig. 23. *Thespesia populnea*. Longitudinal section through the nucleus and perinuclear zone at a stage following the arrangement of the thirteen globular bodies in a plate on the equatorial plane. The chromosomes are massed together at the centre of the nucleus. The spindle is multipolar. At its top left-hand pole is seen the crater of a vacuole.

Fig. 24. *Thespesia populnea*. A transverse section through the perinuclear zone and nucleus at a stage near that shown in Fig. 23. The chromosomes are, either, being drawn to the centre where they will form a mass; or, they may be just coming out of such a mass.

PLATE XXXII.

Fig. 25. *Thespesia populnea*. Eight chromosome masses are seen prior to their arrangement in an equatorial belt upon the spindle.

Fig. 26. *Thespesia populnea*. Cell with its nucleus at the metaphase of heterotype division. Four chromosome masses are seen at one focus upon the equator of the spindle.

Fig. 27. *Thespesia populnea*. The other four chromosome masses of the above as seen on changing the focus.

Fig. 28. *Thespesia populnea*. Anaphase of heterotype division. The section does not pass through the spindle in a truly vertical plane, which accounts for its somewhat smaller appearance than in Fig. 26.

Fig. 29. *Thespesia populnea*. Heterotype division. Three chromosomes lagging behind the rest of the chromatic material which has reached the poles of the spindle and there formed fused masses.

Fig. 30. *Thespesia populnea*. Heterotype division. The chromosomes have reached the poles of the spindle. Three can be seen situated apart from the rest at the upper pole of the figure. Treated with acetic acid.

Fig. 31. *Thespesia populnea*. Anaphase of heterotype division. The chromatic material is moving towards the poles in plate-like masses.

Fig. 32. *Thespesia populnea*. Heterotype division. An oblique transverse section across the spindle cone near the apex and seen from below. Ten club-shaped bodies are seen hanging towards the equatorial plane.

Fig. 33. *Thespesia populnea*. Heterotype division. Ten chromosome masses seen at the apex of the spindle cone; viewed from the equatorial plane.

Fig. 34. *Thespesia populnea*. Heterotype division. The apex of the spindle cone seen in ventro-side view. Three chromosomes lying to one side of an indefinite nodular mass (shown in outline). Treated with acetic acid.

Fig. 35. *Thespesia populnea*. Heterotype division. The chromosomes at telophase as seen at opposite poles of the spindle in one and the same cell. $\times 1,710$.

Fig. 36. *Thespesia populnea*. A group of chromosome bodies seen at the pole of the spindle at telophase of heterotype division. At their ends they show dot or line-like edges indicating probably the tips of the portions composing them.

Fig. 37. *Thespesia populnea*. One of two nuclei at interkinesis following heterotype division. The larger bodies are nucleoli; the rest of the chromatic material is in the form of scattered granules. A nuclear membrane is now beginning to reappear.

Fig. 38. *Thespesia populnea*. Interkinesis. The outer fibres between the two nuclei are derived from the perinuclear zones; the inner ones are apparently remains of the old spindle fibres.

Fig. 39. *Thespesia populnea*. Interkinesis. A nucleus during the reconstruction period.

Fig. 40. *Thespesia populnea*. Interkinesis; reconstruction complete. The two sister nuclei formed by heterotypic division are seen now containing the chromosomes in cross- and rod-shaped forms. The chromosomes are situated peripherally at different foci. This gives the appearance of some crosses appearing in the perinuclear zone; they are really beneath it. Acetic acid fixation.

Fig. 41. *Thespesia populnea*. Two cross-shaped chromosome bodies at the completion of interkinesis. $\times 2,280$.

Fig. 42. *Thespesia populnea*. Early prophase of homoeotypic division. The equatorial plates of two sister nuclei. Ten and thirteen chromosomes are seen in each respectively. Of the ten some have started to divide.

Fig. 43. *Thespesia populnea*. Homoeotypic division, late prophase. In the left nucleus a bipolar spindle has formed, but the chromosomes have not yet arranged themselves upon the equator.

Fig. 44. *Thespesia populnea*. An irregularity. In the same anther loculus as the cell last figured. The chromosomes have obviously failed to separate at heterotypic 'division'. There is a small empty nuclear cavity surrounded by a perinuclear zone. The chromosome bodies are joined together by thread-like connexions into chains of five, and three bodies are separate.

PLATE XXXIII.

Fig. 45. *Thespesia populnea*. Homoeotypic division. An equatorial plate and a spindle.

Fig. 46. *Thespesia populnea*. Homoeotypic division. The chromosome complement seen on the spindle as eight masses.

Fig. 47. *Thespesia populnea*. Homoeotypic division. A nucleus showing anaphase in the case of the original eight equatorial bodies. Five are seen upon the spindle at one focus undergoing division. Three other bodies to be found on altering the focus are shown to one side of the drawing.

Fig. 48. *Thespesia populnea*. Homoeotypic division. Anaphase of the nucleus that contained ten chromosome bodies upon the equatorial plate. The dark bodies are seen at one focus, and those shown in contour at another.

Fig. 49. *Thespesia populnea*. Homoeotypic division. Telophase. The double contour around the outside of all indicates the surrounding hyaline layer. This has been present throughout from the origination of the cell in which heterotypic division occurred.

Fig. 50. *Thespesia populnea*. The fourth nucleus of the previous figure; seen in the next section.

Fig. 51. *Thespesia populnea*. One of the four nuclei of another developing tetrad similar to that shown in Fig. 49.

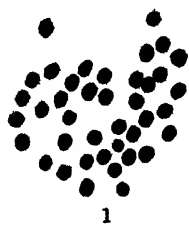
Fig. 52. *Thespesia populnea*. Homoeotypic division. One nucleus has failed to develop to the same phase as the other, being still in early prophase, whilst the other is in anaphase. Such a prophase nucleus grows abnormally large. Five cross-shaped chromosome bodies are seen in it, one being upon the central nucleolus; and three rod-shaped bodies are present.

Fig. 53. *Thespesia populnea*. A developing tetrad, showing three of its nuclei. Each chromosome is in the form of some three granules upon a delicate fibre-like stalk. A nuclear membrane is now present.

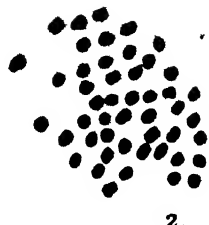
Fig. 54. *Thespesia populnea*. A developing tetrad. $\times 460$. It is enclosed within the hyaline envelope, ingrowing-like secretions of which are now deposited between the separating cells. Scattered granules are now seen in the nuclei.

Fig. 55. *Thespesia populnea*. Section through a tetrad in which all four pollen grains have now separated and are enclosed each within a separate cavity of the hyaline sphere. Three only of the four grains are seen. $\times 460$.

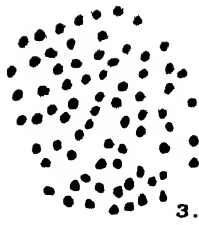
Fig. 56. *Thespesia populnea*. *a* and *b*. At *a* are seen from different nuclei at heterotype division prophase two double bow-like segments which correspond to the figure-of-8 segments, but are without the cross-bow at the centre. At *b* are a double bow and a straight thread with a fibril attached. All are in the same nucleus.



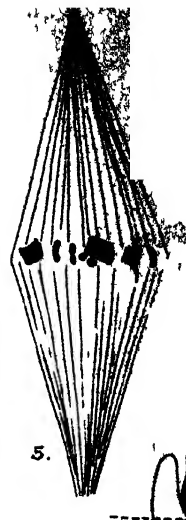
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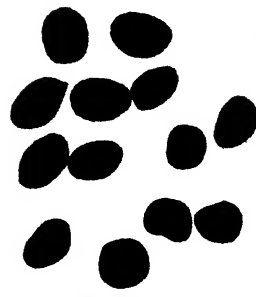
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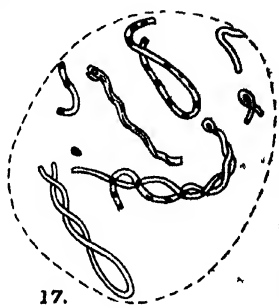


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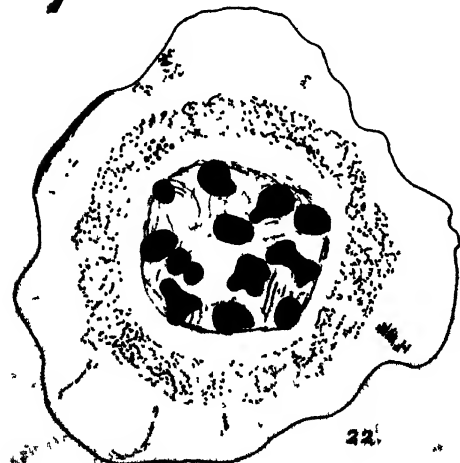


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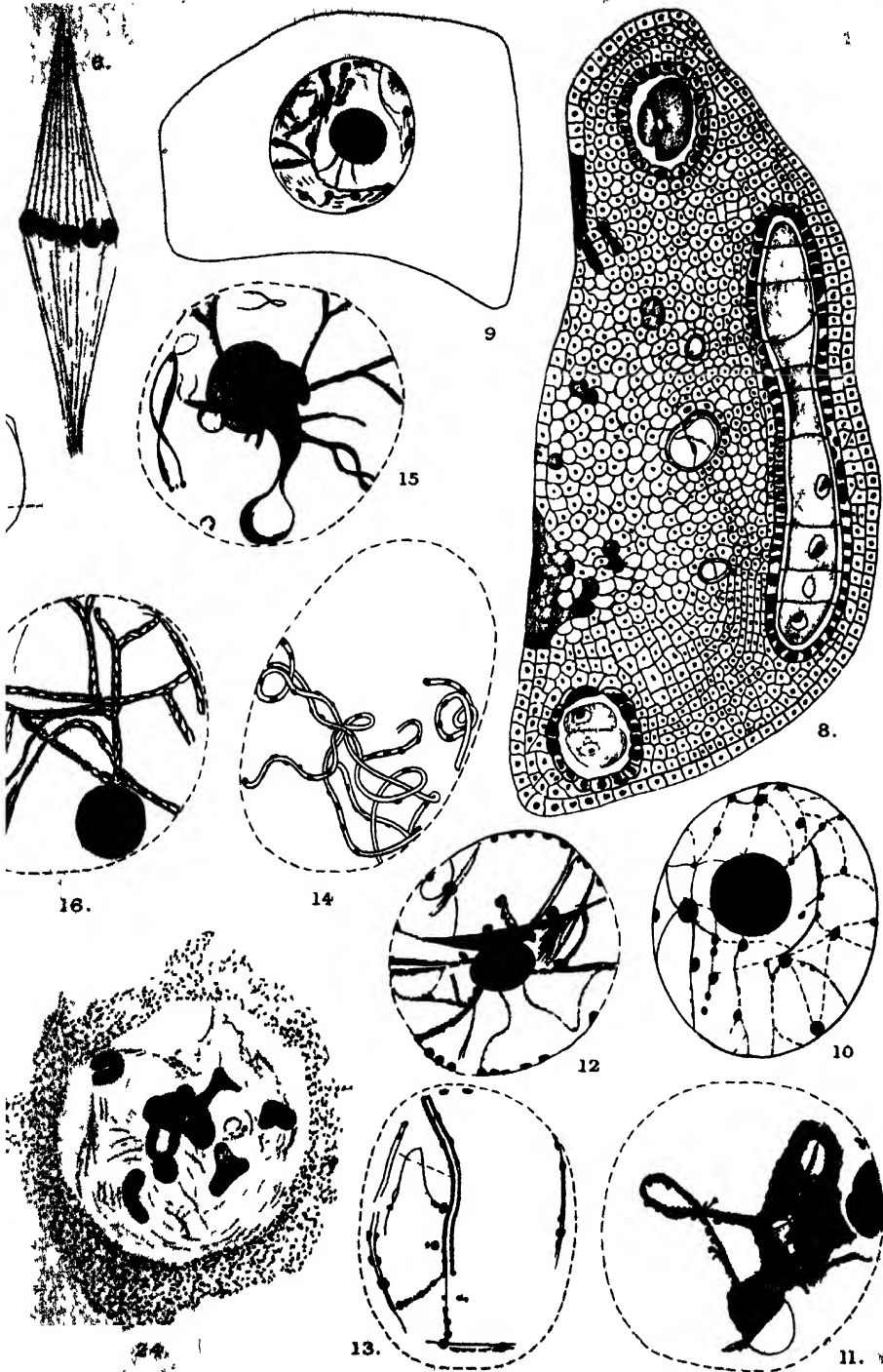
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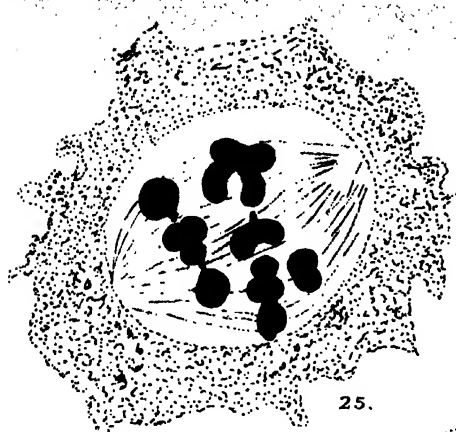


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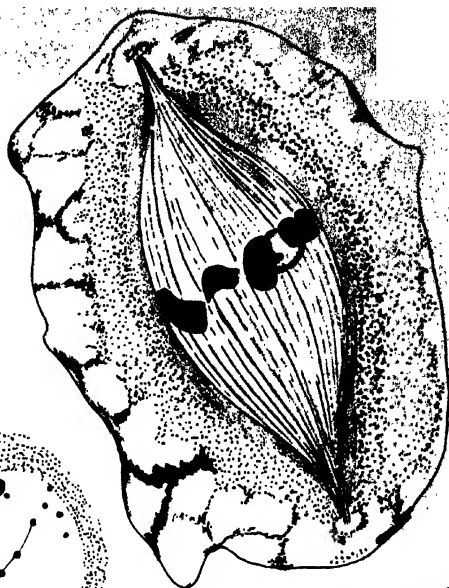


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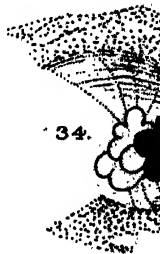
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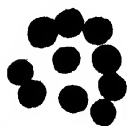
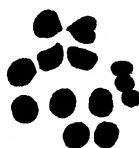
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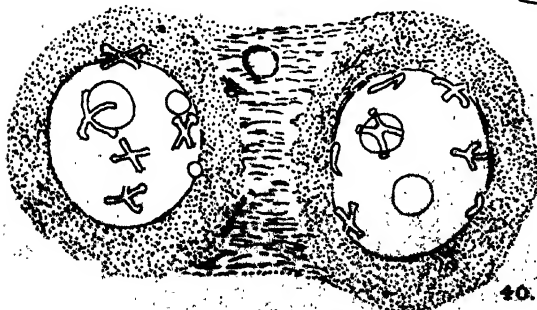
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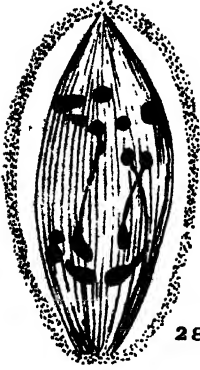
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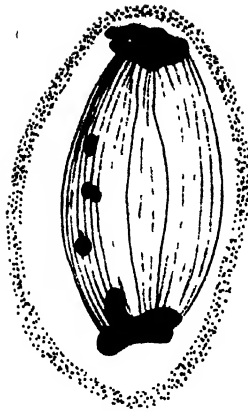
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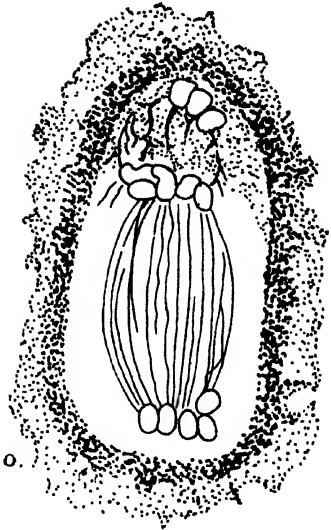
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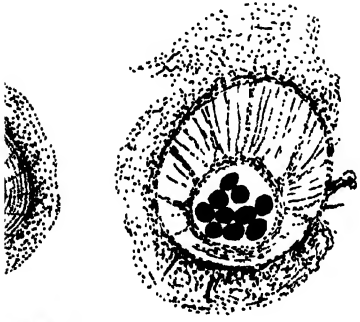
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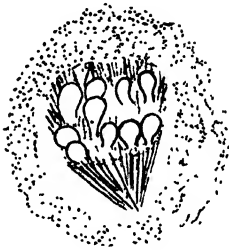
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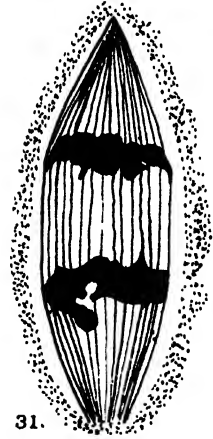
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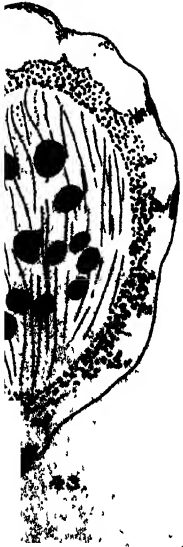
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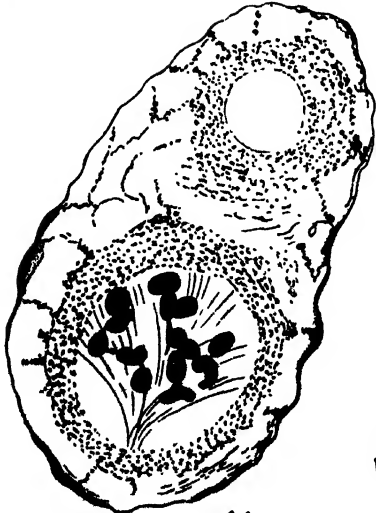
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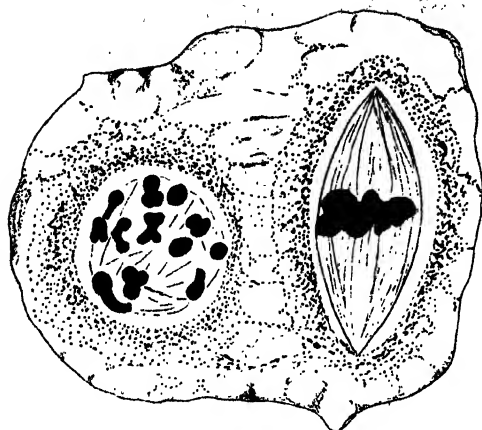
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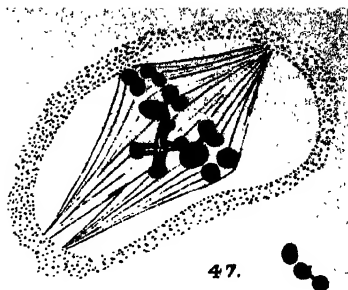
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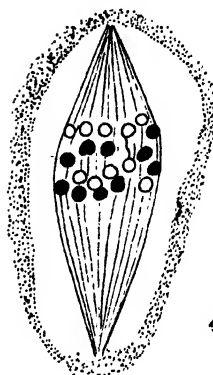
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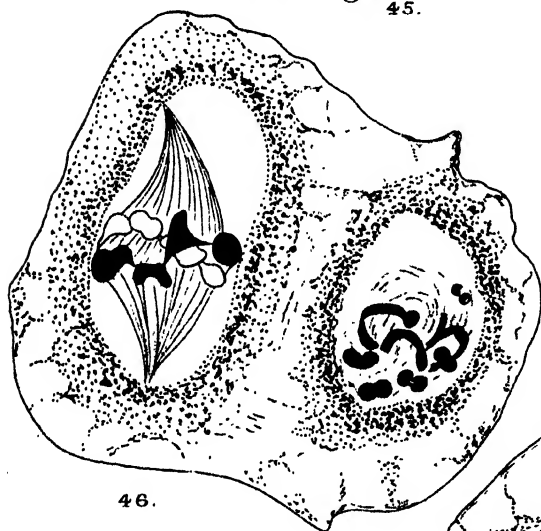
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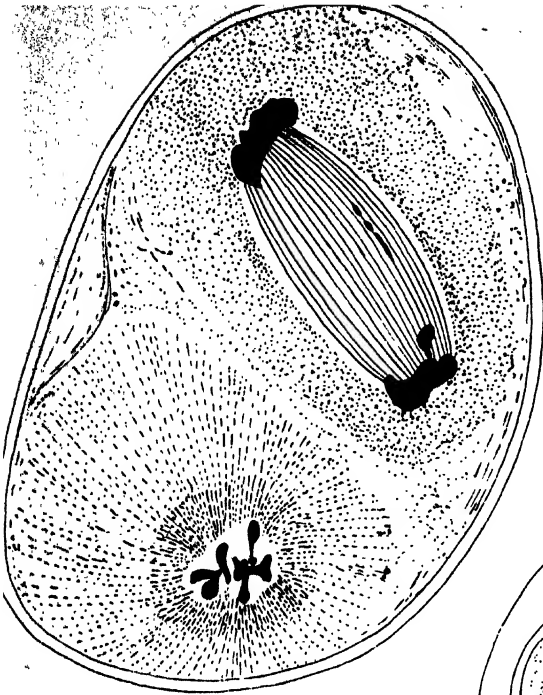
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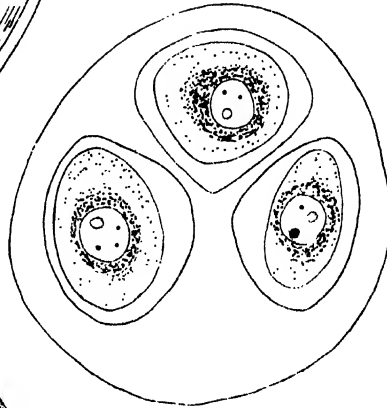
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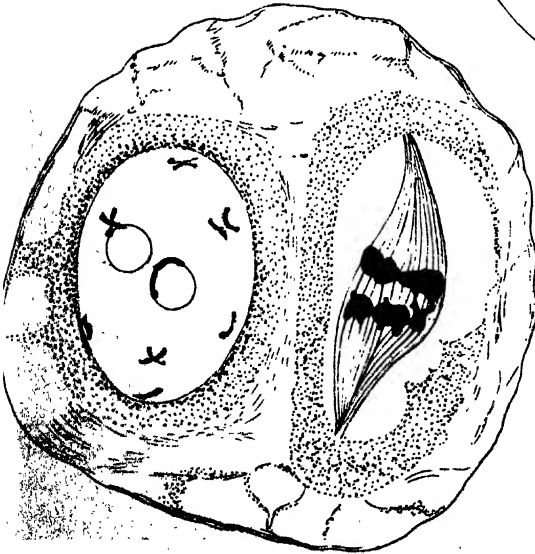
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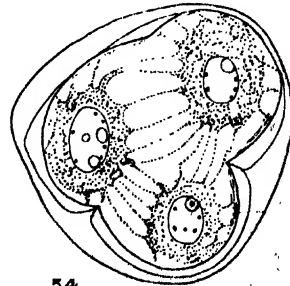
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Cytological Studies of certain Meiotic Stages in *Oenothera*.¹

BY

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With Plates XXXIV-XXXVI and three Figures in the Text.

INTRODUCTION.

OUR knowledge of the genetical behaviour of the genus *Oenothera* was for some years in a rather confused state. The earlier cytological studies of the genus made by Gates, Davis, and others did to some extent elucidate the problems, but still the extraordinary behaviour in inheritance of many of the species was not satisfactorily explained. The behaviour of the chromosomes during meiosis was studied in several pure species, hybrids and mutants: in practically all these forms the salient features of reduction division were found to differ materially from those characteristic of the reduction phases in other Angiosperms. The early prophase stages of the nucleus of the pollen mother-cell were quite comparable to those occurring in other genera. The chromatin thread after synizesis loosened again; then it underwent a second contraction immediately prior to diakinesis. During the stage which corresponds to diakinesis the majority of the chromosomes were usually found not to be paired; occasionally a few pairs were seen, but the rest of the univalents lay in the nuclear cavity either separately or joined together in longer or shorter chains, having remained attached telosynaptically as they had been on the spireme. After the spindle was formed and had become bipolar, this irregular arrangement still persisted. No regular metaphase plate was formed. At anaphase the members of any bivalents which had been formed passed to opposite poles, but it appeared to be entirely a matter of chance to which pole any particular chromosome passed. *O. grandiflora* was the only species whose behaviour at reduction division at all resembled that which appears to be typical of the average Angiosperm (17). Certain abnormal phenomena observed in the genetical behaviour were thought to be due to 'crossing over', but no cytological basis for this idea was forthcoming.

¹ Thesis approved for the degree of Master of Science in the University of London.

Recently, however, further cytological investigations of the genus have been carried out in America by Cleland, and in Europe by Oehlkers (46) and Håkansson (33). Altogether some thirty or forty pure species, hybrids, and mutants have been examined. In practically all of these, certain striking arrangements of the chromosomes become evident during late heterotypic prophase and persist after the nuclear membrane has disappeared and the multipolar spindle is formed. These arrangements are usually shown as a constant feature by any one form, each form having its characteristic configuration. Further, it is now found that whether or not pairing of homologous chromosomes occurs, a mechanism exists ensuring their regular separation at heterotypic anaphase and the consequent regular segregation of germinal factors at each reduction division.

This paper will deal essentially with the heterotypic divisions of the pollen mother-cell nuclei of some five species of *Oenothera*. Of these, four forms show such arrangements in 'diakinesis' and 'metaphase' fairly constantly, but the fifth, *O. Agari*, exhibits many anomalies.

MATERIAL.

All the material used was obtained from genetical cultures grown in the Royal Botanic Gardens, Regent's Park, by Professor Ruggles Gates.

O. novae-scotiae. This species was described originally as a segregate from *O. muricata* (26) and has bred pure for many years.

Two different strains of the species and also an early type of one of them were examined cytologically. In each case material was collected from several different plants of each of several cultures. Collections were made in July, August, and September in 1925 and 1926. All the material gave identical cytological results.

O. eriensis. Seed of this species was obtained in August, 1924, from plants growing at Colchester on Lake Erie. It was grown in cultures the two following years. Numerous plants were grown and were all remarkably uniform in appearance (28).

Material for cytological study was collected in July and September in 1926 from six different plants, all of which gave the same results.

O. ammophila, Focke. The strain examined is naturalized in Heligoland. Seed was sent by Professor Renner to Professor Gates and was grown at Regent's Park Botanic Gardens. Unfortunately only one plant germinated in 1926. Several collections of material were made from this, however, in July and September.

O. rubricalyx. This form first appeared as a mutant in a culture of *O. rubrinervis* in 1907, since when it has been carried on in a pure line (24). Material was collected in August and September, 1926, from several different plants, all of which gave identical results.

O. Agari. The species was first found on the shores of Lake Burnie, in Tasmania, by Professor Agar in 1923. During three years it has been grown in cultures, and with the exception of one narrow-leaved mutant, which appeared in 1925, it has been of extremely uniform appearance and behaviour.

Cytological material was collected in 1924 and 1925; all that examined gave similar results.

METHODS.

Material was collected between 11.30 a.m. and 2.30 p.m. on warm sunny days. Buds of varying sizes were fixed; in the largest the anthers were just turning yellow, indicating that the pollen was fully formed. In order to facilitate penetration of the tissues by the fixative, the sepals were always removed and an exhaust pump was used.

The fixatives used throughout were:

1. A chrome-acetic solution containing:

Chromic acid	1 gram.
Glacial acetic acid	1 c.c.
Water	100 c.c.

Buds were fixed for two days and were then washed in running water until they were colourless, after which they were dehydrated by running through a series of alcohols and were cleared in xylol.

2. One of Allen's modifications of Bouin's fluid, which was made up as follows:

Picric acid, saturated aqueous solution	75 c.c.
Formaldehyde, 40 per cent.	25 c.c.
Glacial acetic acid	5 c.c.
Urea	2 gram.
Chromic acid	1.5 gram.

The liquids were mixed and heated to a temperature of 38° C. and the two solids were then added. The fluid was allowed to cool gradually while the collection was being made. Owing to the distance of the laboratories from the Botanic Gardens, it was not possible to keep the time of fixation constant. It varied from one to about three hours, but this apparently had no deleterious effect on the general fixation. The material was run up to 70 per cent. alcohol in one hour; it was then washed thoroughly in 70 per cent. alcohol containing a few drops of lithium carbonate. Alcohol or aniline oil was used to dehydrate the material and xylol for clearing. The buds were embedded in paraffin wax and sectioned at a thickness of 10 or 12 μ . The modified Bouin's fluid appeared to give the best results.

Various stains were tried, including safranin and gentian violet, iodine-gentian violet (9), Coles's rapid iron-haematoxylin (16), and Heidenhain's iron-alum-haematoxylin. The last mentioned gave by far the best results.

I am indebted to Dr. J. Latter, who fixed all the material collected in 1924 and 1925.

DESCRIPTION.

The resting and early prophase stages of the pollen mother-cell nuclei are almost identical in all those species studied and with similar stages previously described as occurring in other forms (10, 14, 17, 18, 19, 23). This work was not primarily intended to be a study of synapsis, but as some additional details have been observed, a general outline of the early development of these five species will be given, the figures being selected from the different species. In these forms all the pollen mother-cells of a single loculus develop almost simultaneously, and usually little difference is seen in the stage of development reached by the pollen mother-cells of the different loculi of a bud.

The Resting Nucleus of the Pollen Mother-Cell.

After the last premeiotic division of the archesporial tissue, the pollen mother-cells are seen to be packed closely together within the loculus, most often in a single vertically seriated row. Very occasionally two or more vertical rows are found. They are distinctly polygonal in section, there being no spaces between the individual cells, and, except where faulty fixation has caused slight shrinkage, there is no space between the pollen mother-cells and the tapetum.

The resting nucleus of the pollen mother-cell is roughly spherical in shape and has a very thin membrane enclosing it. Towards the periphery of the cavity is a faintly staining, small-meshed reticulum, the threads of which are very finely granular, larger granules being sometimes present where the threads meet. The centre of the cavity is occupied by a single, large, dark-staining nucleolus, which can sometimes be seen to be vacuolate (Pl. XXXIV, Fig. 1). Occasionally two smaller spherical nucleoli are present.

On further destaining certain of the preparations it was possible to study the contents of the nucleolus. In some cases it appears merely as a faintly staining spherical body which is often vacuolate, a single, large, central vacuole being frequently present. In numerous instances this cavity was found to have definite contents, often being occupied by a single, large, highly refractive, crystal-like structure (Pl. XXXVI, Fig. 1a). These structures vary in shape, the vacuole usually being polyhedral, having assumed roughly the same shape as the body included within it. The inclusion appears to have a strong affinity for chromatin reagents and remains stained when the nucleolus is almost totally decolorized. The crystalloid is too small for

it to be possible to try any specific chemical tests to determine its nature. It is, however, clearly not a simple inorganic waste substance, as such compounds would be dissolved by the fixatives used, and, further, they would not stain with chromatin dyes. This crystalline inclusion within a large vacuole is a constant feature of the nucleolus of the premeiotic resting nucleus of *O. rubricalyx*. In the other species studied it is not of such frequent occurrence. Such a structure has already been described as occurring within or taking the place of the vacuole in the nucleolus of the resting nucleus of the pollen mother-cells of *O. franciscana* (10). A similar body is thought to be a constant feature of the resting nuclei in the pollen mother-cells of *Lathyrus odoratus* (37), the fact that they are not always observed in these nuclei being explained by their peculiar staining reaction. It does not seem to be possible that they are always present at this stage in all those *Oenotheras* studied. Quite frequently one pollen mother-cell may show such a crystalloid, whilst its neighbour, which seems to have reacted to the stain in a precisely similar manner, contains no such body and the nucleolus may even be devoid of vacuoles. This, combined with the fact that they are observed regularly in *O. rubricalyx*, although the intensity of the stain may vary, seems to indicate that they do not occur regularly in the other species.

In the endosperm of *Macrozamia Fraseri* prior to free nuclear division definite crystalline material is present in the nucleoli and appears to be dissolved away during the prophase (39). Similar crystalloids are reported to occur in the nucleoli of the pollen mother-cells at diakinesis in *Zea Mays*, L. (36), and are also found in the resting stage and at diakinesis in the pollen mother-cells of *Lathraea clandestina* and *L. squamaria*. During the resting stage of the root-cell nuclei of *Allium Cepa* the nucleoli often contain crystals which do not stain with chromatin dyes and which are apparently absorbed during the development of the spireme (48). One or more 'curious crystalline-looking bodies' are often present in the nuclei of the two or three outer rows of cells of the root of *Galtonia candicans* (20). These structures are apparently not identical with the others mentioned as, although probably derived from the nucleolus, they lie free within the nuclear cavity.

Early Prophase Stages.

When the nucleus again becomes active at the beginning of meiotic division, the structure of the nucleolus is altered considerably. In those cases where a large crystalloid is present it gives off successively a number of smaller ones, becoming itself greatly diminished in size (Pl. XXXIV, Figs. 2a, 2b), and may become indistinguishable from its derivatives. The nucleolus often comes to contain as many as a dozen small crystalloids, each lying within its own vacuole. The vacuoles may be connected with each other, the

interior of the nucleolus thus becoming honeycombed. About this time the nucleolus itself may bud (Pl. XXXIV, Fig. 2*a*), a smaller nucleolus being given off (8, 54). When formed, this latter persists throughout the earlier meiotic phases (Pl. XXXIV, Fig. 3). The crystalloids and their surrounding vacuoles then gradually disappear, and the nucleolus becomes homogeneous in nature. In practically all cases the nucleolus passes towards the periphery of the cavity (Pl. XXXIV, Figs. 2 and 3), but there is apparently no connexion between the direction taken by the nucleoli in the cells of the same locus; all nucleoli seem to move independently.

During this time the fine network of the resting nucleus has given place to a much-simplified reticulum. It would appear that some threads have been absorbed by those neighbouring, giving a coarser large-meshed structure. The threads now comprise relatively dark-staining granules, which lie along a pale-staining thread. In some cases a few very large aggregations of chromatin are formed and lie at the periphery of the cavity. These bodies, like those described in *O. rubrinervis* (23), show no constancy in size, shape, or number, and in most cases disappear quite early. There is nothing whatever to suggest that they are prochromosomes.

Eventually the reticulum becomes completely resolved into a single long, fine, apparently continuous thread (Pl. XXXIV, Fig. 3). During these stages only slight indications of parallelism are found, such as must occasionally occur accidentally; nor are any further indications of parallel threads seen during later stages. The nucleolus has now reached the nuclear membrane, apposed to which it lies, assuming a lensiform shape. The whole of this presynizetic phase is apparently passed over rather rapidly, as only a small proportion of nuclei examined are in this condition.

The nucleolus. As the reticulum gives place to the long, continuous spireme, the latter is seen to be in organic connexion with the nucleolus. Such connexions between the thread and the nucleolus have been described in a number of plants.

When sections are very carefully destained so that the general substance of the nucleolus is left almost colourless, a small dark-staining body is seen to lie within it and to be attached to the chromatin thread. By this time the thread has assumed a slightly greater affinity for chromatin dyes, whilst the nucleolus does not seem to have quite such a great affinity as formerly. If great care is taken with the differential staining of the nuclei, it is always possible to obtain a pale-stained nucleolus containing within it a small dark-staining body which has apparently alone retained the former great attraction for chromatin dyes. Attached to this body is found one or sometimes more loops of the long, tangled spireme thread (Pl. XXXIV, Fig. 3). Very occasionally more than one such dark-staining area is visible within the nucleolus, and in such cases the thread is attached by loops to both bodies. Sometimes more than one nucleolus is present within a single

nucleus. In such a case both behave in a precisely similar manner, the thread being attached to a particular dark-staining area within each. This connexion between the spireme thread and the body within the nucleolus is a constant feature of those five species of *Oenothera* examined. The body is undoubtedly that seen in *Oenothera* species by Cleland (10, 15), who termed it the 'endonucleolus'. In *O. biennis* he finds that the endonucleolus has in some cases organic connexion with the reticulum, but he does not suggest that any significance is attached to the fact. This body within the nucleolus is undoubtedly comparable with the 'nucleolar body' which occurs in the nucleolus of the pollen mother-cell nuclei in *Lathyrus odoratus* (37). Here a single, often spherical, dark-staining body is always found lying at the periphery of the nucleolus; attached to it is a single loop of the spireme thread. In *L. odoratus* the nucleolar body is apparently superficial in position and is often seen projecting from the nucleolus as a small papilla. In *Oenothera*, although the body appears to lie towards the periphery of the nucleolus, it does not usually project from the surface. Except for a small area at which the thread is attached to it, the body seems to be embedded within the otherwise homogeneous substance of the nucleolus. Thus the terms 'endonucleolus' and 'nucleolar body' would be equally applicable. For convenience, the former term will be used.

This endonucleolus of *Oenothera* appears to be usually considerably smaller than the nucleolar body found in the nucleoli of *Lathyrus odoratus*. In the former case it is often spherical; it may, however, take a variety of forms, and may be of a considerable size (Pl. XXXIV, Figs. 5-12). A similar variously shaped structure, to which the thread is attached, has recently been found to occur constantly in the nucleoli of the pollen mother-cell nuclei of *Lathraea clandestina* and *L. squamaria* (unpublished work of Gates and Latter). In *Oenothera*, *Lathraea*, and *Lathyrus* the endonucleolus or nucleolar body persists throughout the greater part of the prophase, remaining all the while in organic connexion with the spireme.

Synizesis. The spireme thread gradually thickens, and consequently shortens, and is also becoming more easily stainable, while the nucleolus is rapidly becoming less so. This suggests strongly that material is being transferred from the nucleolus to the thread. The spireme then contracts, and all the loops are drawn into a tightly tangled, deep-staining knot, from the edge of which a few loops may project, but no portion of the thread can be traced for any appreciable distance (Pl. XXXIV, Fig. 4). This knot lies quite close to the nucleolus, often obscuring the endonucleolus (Pl. XXXIV, Figs. 5-7). This behaviour contrasts with that of *Lathyrus odoratus*, where the knot draws away from the nucleolus, tending to lie on the opposite side of the cavity. However, in this case separation is never completed, a single loop projecting always from the knot and connecting it with the nucleolus. In *Oenothera*, conversely, the synizetic knot and the nucleolus together occupy

only a very small portion of the nucleolar cavity. Judging from the large number of nuclei found in this condition, it would appear that synizesis takes a relatively long period of time.

During the presynizetic and synizetic stages the behaviour of *O. novae-scotiae* differs in some respects from the species previously described. During early synapsis large, variously shaped masses of chromatin appear within the now simplifying reticulum. Such aggregations do occur in other species, but do not usually assume quite such an irregular form as here (Pl. XXXIV, Figs. 10, 11). In *O. Agari*, *O. eriensis*, *O. rubricalyx*, and *O. ammophila* these masses disappear quite early, being apparently absorbed by the thread, which usually becomes comparatively smooth at a quite early stage (Pl. XXXIV, Fig. 3). In *O. rubrinervis* (23) they persist for a longer time, being swept into the synizetic knot, and are apparently absorbed there. In *O. novae-scotiae* such large rounded masses, which are often connected into a kind of chain (Pl. XXXIV, Fig. 12), persist until synizesis and can be easily observed with a certain depth of stain. A possible explanation of the late occurrence and unusual appearance of these will be suggested later.

The phenomenon of *cytomyxis* has been observed in these species in a number of instances during early prophase, and especially during synizesis. Cytomyxis occurs as an abnormality in a number of plants; the literature dealing with the subject is quoted fully by Gates and Rees (29). In the present instances either isolated cells or several cells of a loculus or the whole loculus may be affected. When the nucleus of a pollen mother-cell becomes so eccentric that its membrane is in contact with the cell-wall, the nuclear contents tend to flow through the pores of the cell-wall, which are normally occupied by plasmodesmae, into the adjacent pollen mother-cell. When a number of adjacent pollen mother-cells are affected, the nuclei all flow in the same direction. After synizesis the pollen mother-cells draw apart from each other, rendering the process impossible. The phenomenon is found in comparatively well-fixed material, but is of much more frequent occurrence when fixation is poor. When cells are thus affected they must either degenerate, in which case some residuum would be found in later phases; or they must continue to develop, when their development would be of necessity abnormal. However, when preparations showing later stages in the development of the pollen mother-cells are examined, no indication of the phenomenon is found, no abnormalities which might have arisen through cytomyxis are observed, nor are any cells seen which appear to have ceased their development thus early, and from this cause. These facts suggest that cytomyxis may be an artifact due to the fixation or subsequent treatment of the material.

Open spireme. While the loops of the thread are tightly woven together within the synizetic knot, the spireme continues to thicken. When

the loops unfold, tending again to fill the cavity, the spireme is considerably thicker and much shorter (Pl. XXXIV, Figs. 8, 9). The pachynema is usually fairly smooth in outline, but occasionally it is found to be moniliform. The especially thickened areas which were earlier observed in *O. novae-scotiae* have now completely disappeared, their substance having presumably been absorbed into the rest of the thread.

During all the prophase stages the pollen mother-cell and the nucleus contained within it have been steadily increasing in size; the latter seems to attain its maximum diameter during synizesis or open spireme stage. This, of course, is not clear from the figures, as they have been selected from four or five different species which differ in size among themselves, and also some slight variation is shown by a single species.

During open spireme the thread continues rapidly to shorten and increase in thickness. This whole process is presumably over very quickly, as the stage is very difficult to find in the preparations. A late stage in open spireme, where the pachytene thread is about to undergo second contraction, is seen in Pl. XXXIV, Fig. 13, of *O. eriensis*. Here the whole thread can be traced as being single and continuous, although some indication of the beginning of segmentation is observed. Similar stages occurring in *O. Agari* are illustrated in Pl. XXXVI, Figs. 55 and 56.

Second contraction then occurs, the now relatively thick thread being drawn into another tight knot (Pl. XXXIV, Figs. 14, 15, 27, and Pl. XXXV, Fig. 34). This second contraction occurs in *Oenothera* as constantly as does synizesis (10, 12, 14, 23), but it is not usually of such long duration. Second contraction does not occur at precisely the same stage in the thickening of the spireme in all *Oenothera* species. Davis (19) describes it as occurring after the segmentation of the spireme, but other workers find that usually segmentation begins whilst the thread is in its contracted state. Usually it is of short duration, but this also varies slightly in different species. For instance, in *O. franciscana* it is a relatively slow process (10). In *O. eriensis*, *O. Agari*, and *O. novae-scotiae* a relatively thickened pachynema, which is beginning to show some indications of segmentation, is observed in open spireme (Pl. XXXIV, Figs. 13; Pl. XXXVI, Fig. 56), but segmentation into fourteen univalents is by no means complete until after the knot is formed. In these species the contracted phase is accomplished quite rapidly. In *O. rubricalyx* a slightly thinner thread, showing no indication of segmentation, undergoes the second contraction, from which it emerges as a much-thickened, fully segmented thread. In *O. ammobhila*, Focke, a still finer thread enters the knot, and here again a completely segmented thread emerges. As would be expected, second contraction in these two latter species lasts for a relatively longer period. In no case does the time expended in second contraction in any way approach that occupied by synizesis, however.

The second contraction phase is not typical of all Angiosperms, although it has been described in a number of plants, including *Lilium*, *Podophyllum*, *Tradescantia* (44), *Eucharidium concinnum* (52), *Daphne pseudo-mezereum* (47), and *Ranunculus acris* (54). In *Lathyrus odoratus* (37) second contraction is replaced by a brochonema stage, where a number of loops equal to the haploid number (seven) of chromosomes radiate out from a central tangle, which is gradually used up in the development of the loops. In those species described by Mottier no thickening of the spireme apparently takes place during this contracted phase; this is not, however, the case in other genera, including *Oenothera*, where considerable shortening and thickening occurs during this stage. The chromatin thread is still attached to the endonucleolus, which is now the only part of the nucleolus retaining anything like its former capacity for chromatin stains.

Later Prophase Stages: 'Diakinesis.'

On emerging from the second contraction knot, the thread is seen to have become constricted into a number of pieces equal to the diploid number of chromosomes, which in all cases studied was fourteen. The thread has at last broken away from the nucleolus, and the endonucleolus has disappeared. The nucleolus itself still remains usually as a very pale-staining biconvex body lying usually against the nuclear membrane. It is always present in 'diakinesis': it has, however, not always been figured, as in some cases the chromosomes would have been obscured. Occasionally a dark-staining nucleolus is found at this time, but this is unusual. The individual chromosomes which have just become apparent are several times as long as they are broad, and their outline is far from smooth. At this time they are probably of a spongy texture (Pl. XXXV, Fig. 37, and Pl. XXXVI, Fig. 59). Soon they become smooth in outline and continue to contract in size throughout 'diakinesis'. It is now that the characteristic configuration of 'diakinesis' is first clearly seen, and removes all doubt as to the mode of synapsis within the genus. It is rare to find all the bivalents paired in 'diakinesis', although this does occur in a few species, e.g. *O. Hookeri* (51), *O. grandiflora* (17), and in the mutants *O. blandina* and *O. deserens* (13). Much more often the attraction between the telosynaptically arranged chromosomes is as great as that existing between homologues. Thus, when the newly formed chromosomes emerge from the second contraction knot, it is not usual to find that they are paired, but they are generally arranged in rings consisting of varying numbers of univalents. The number of univalents contained in each ring is fairly constant throughout any given species. In some cases one or more pairs of univalents are constricted off, whilst the other chromosomes remain attached end to end, as they were on the spireme. In the different species which have been described all

possible arrangements from complete pairing to its total absence have been observed.

O. eriensis. When the spireme emerges from the second contraction knot, it is found still to consist of a single continuous thread (Pl. XXXIV, Figs. 14, 15), now, however, much shortened and constricted into fourteen portions or chromosomes. These are several times as long as they are broad, and seem to be of a spongy texture. They may lie quite close together, or they may lie at some distance from each other, being attached together in a continuous chain by extraordinarily long, fine, faintly staining threads (Pl. XXXIV, Figs. 16, 17). The ring of concatenated chromosomes is still much too long to lie stretched out within the nuclear cavity, and consequently becomes thrown into a number of loops. Some hundreds of cells were examined; in some few of these the regular arrangement of the fourteen chromosomes in a single ring is not observed, the circle often being broken up, the univalents lying within the cavity either singly or joined together into longer or shorter chains, giving an appearance similar to Pl. XXXIV, Figs. 22, 23. Of course, in many cases the nucleus must be cut by the microtome knife, some chromosomes being removed. The scattered appearance, however, occurs also when all the chromosomes are present. When we consider how very long and fine are the threads between the univalents, it seems most probable that this apparent anomaly is either an artifact or is due to imperfect staining. It is likely that these filiform connexions would easily be completely broken down either by faulty fixation or by subsequent treatment. It is also likely that if the stain is not perfectly differentiated, the connexions would be completely invisible. In such a case it would be impossible to judge which chromosomes are really adjacent, owing to the distance between them, and they would give a very scattered appearance. In no case was a closed ring containing less than fourteen chromosomes found. It seems to be quite certain that a closed chain of fourteen chromosomes is a natural and constant feature of 'diakinesis' in this species.

During 'diakinesis', which lasts for a comparatively long time, the individual chromosomes continue to contract in size until they become much smaller and have a smooth surface. This contraction is not uniform among all the chromosomes, and hence univalents of very different sizes often lie adjacent in the same nucleus.

O. novae-scotiae. In this species the arrangement of the chromosomes during 'diakinesis' is essentially the same as that found to occur in *O. eriensis*, in that all fourteen univalents are arranged in a single closed chain which becomes looped and contorted within the nuclear cavity (Pl. XXXIV, Figs. 20, 21). A similar arrangement is shown by *O. muricata* (see (15)), which belongs to the same small-flowered group as *O. novae-scotiae*. *O. novae-scotiae* differs from *O. eriensis* in the fact that in the former the

neighbouring chromosomes of the ring lie much closer together, the connexions between them being shorter, not usually so fine, and often showing a greater affinity for chromatin dyes.

Some hundreds of cells in this stage from two different strains and many different collections were examined, and when a nucleus was uncut it practically always contained a ring of fourteen chromosomes. In a very few cases scattered arrangements were seen (Pl. XXXIV, Figs. 22, 23), but these were not of such frequent occurrence as in *O. eriensis*, their frequency in the latter species being probably due to the long, fine connexions between the chromosomes. In one case only was an exceptional arrangement found in *O. novae-scotiae*. Pl. XXXIV, Fig. 24, shows a ring of six linked chromosomes, the other eight being apparently scattered. The typical configuration of the chromosomes in 'diakinesis' is again a ring of fourteen. As in *O. eriensis* the univalents continue to contract during 'diakinesis' and lose their spongy nature.

O. ammophila, Focke. This species differs from those described above in the appearance presented by the nuclei in 'diakinesis', but resembles *O. Lamarckiana* (13, 33) and certain of its mutants (33), also *O. franciscana sulfurca* (12), and certain crosses described by Oehlkers (46). In these species a large ring of chromosomes and a very small one are present in the nuclear cavity in 'diakinesis'; the two rings may be linked together or may lie quite separately. The smaller ring consists of a single bivalent, the two chromosomes which comprise it being joined at both ends, whilst the larger comprises the other twelve chromosomes (Pl. XXXIV, Figs. 30, 31). In the material examined only one exception to this configuration was found. In this genus the arrangement characteristic of 'diakinesis' persists long after the disappearance of the nuclear membrane. In one pollen mother-cell of *O. ammophila* a single closed chain of fourteen chromosomes was found lying on the multipolar spindle (Pl. XXXIV, Fig. 33). As this was the only exceptional case among numerous cells, it may safely be assumed that the normal arrangement of the chromosomes in 'diakinesis' is in a circle of twelve with a single pair separated from it and bent round to form a small ring.

As in the other species examined the chromosomes become more and more compact during the process of 'diakinesis'.

An attempt was made to find out at exactly what stage the pair becomes cut off from the rest of the spireme, if indeed it is ever linked to it. During the early prophase stages there is nothing whatever to indicate that one pair is segmented from the other six. An examination of a number of nuclei in the open spireme stage yielded no really satisfactory results. Owing to the relatively early stage at which the thread enters the second contraction knot, it is impossible to trace the open spireme throughout its entire length, as can occasionally be done in other species (Pl. XXXIV, Fig. 13 ;

Pl. XXXVI, Figs. 55, 56). There is nothing whatever in the arrangement of the loops of the open spireme to suggest that its construction is in any way different from that of *O. novae-scotiae* or *O. eriensis*. The first indication of the separation of a small portion of the spireme from the main length is shown in Pl. XXXIV, Fig. 27, which illustrates the nucleus soon after the spireme has entered the second contraction knot. A short length of thread, presumably representing a single bivalent, has been cut off from, and is now interlinked with the rest of the pachynema. Apparently this pair may break away from the rest of the spireme quite early (Pl. XXXIV, Figs. 28, 29), or it may remain linked even on to the spindle. It is quite possible that in some cases it breaks away from the larger ring when it is first cut off.

O. rubricalyx. In 'diakinesis' in this mutant the chromosomes are normally arranged in a ring of six together with four bivalents. The chromosomes of the bivalents are bent round and joined at either end to form small rings. In 'diakinesis' the bivalents may be linked to the large ring or to each other, or they may lie quite free within the cavity (Pl. XXXV, Figs. 35-9). Two exceptional configurations were found lying in the nuclei of two adjacent pollen mother-cells. In both these cases a ring of four chromosomes and five separate ring pairs were found (Pl. XXXV, Fig. 40).

As is typical of the genus the spongy nature of the chromosomes is lost during 'diakinesis', since they still continue to contract.

Here, again, an attempt was made to determine whether or not the pairs are cut off from the main part of the spireme during the prophase, and, if so, at what precise stage they become cut off. Again, no evidence could be obtained from the open spireme stage, which appeared precisely like that of the other species studied. During second contraction, however, a stage was found which seems comparable to the brochonema stage of *Lathyrus odoratus* (37). Four small loops project from a central tangle of thick threads (Pl. XXXV, Fig. 34). These are without doubt the initials of the four bivalents which become evident in 'diakinesis'. It would thus appear that in *Oenothera* when pairs are formed they are cut off from the spireme at about the same stage that the spireme segments into a number of bivalents in other plants. In *O. ammophila* and *O. rubricalyx* the pachytene thread does not begin to segment until it has entered the second contraction knot. It is evident that the pairs are cut off early in second contraction; but it is not possible to determine whether they are cut off before or after the general constriction of the thread into fourteen segments occurs or whether the two processes take place simultaneously.

During 'diakinesis' a number of fibrillae appear in the cytoplasm around the nucleus. They seem to radiate from several points in the cytoplasm and to run in a direction tangential to the nuclear membrane. The membrane is then quite rapidly dissolved and the fibres of this multipolar spindle

penetrate into the cavity. The nucleolus, which has completely lost its affinity for chromatin dyes and during 'diakinesis' appears as a pale homogeneous body, disappears quite suddenly at the same time as the nuclear membrane. It does not first become vacuolate and does not fragment. No signs of nucleoli are found either on the multipolar spindle or in the cytoplasm surrounding it. In all these species the chromosome configuration so strikingly characteristic of 'diakinesis' persists unchanged on to this multipolar spindle. The chromosomes now, however, have become much more dense and are consequently a mere fraction of their original size (Pl. XXXIV, Figs. 19, 25, 26, 32; Pl. XXXV, Fig. 35). Despite this condensation they do not break apart, but the large rings become much smaller and are not usually so contorted as they were in 'diakinesis'.

O. Agari. In the majority of species which have been described each is characterized by a special arrangement of the concatenated chromosomes which first becomes apparent as the second contraction knot unfolds and persists through 'diakinesis' on to the multipolar spindle. Irregularities in 'diakinesis' have been recorded as occurring in one or two trisomic mutants of *O. Lamarckiana* (33). Beyond these, *O. Agari* seems to be the only species at present known in which a regular arrangement of chromosomes in rings of specified size does not occur in 'diakinesis'. In this species closed rings of chromosomes are formed in 'diakinesis' as a constant feature, but their constitution is far from regular. In a fair proportion of the pollen mother-cells all fourteen chromosomes are joined together in a single continuous chain (Pl. XXXVI, Figs. 57, 58), but in many cases closed rings of smaller numbers of chromosomes are formed. Pl. XXXVI, Fig. 59, shows a nucleus in which four rings are present in 'diakinesis', two of these consisting of three chromosomes each, while the construction of the other two is not quite clear: either the large one consists of five chromosomes and the smaller one of three, or the larger contains six and the smaller is a bivalent. Pl. XXXVI, Fig. 60, is unfortunately cut and shows only twelve chromosomes, but these indicate another alternative arrangement, the larger ring containing eight chromosomes and a ring of three being present also.

In this species, as in all others, the arrangements prevalent in 'diakinesis' persist on to the multipolar spindle. In some cases the connexions between the chromosomes cannot be traced, but their alinement leaves no doubt as to their original arrangement. Pl. XXXVI, Fig. 61, indicates that the chromosomes have been arranged in three rings of four, five, and five respectively, these groups now lying in three distinct focal planes. Only one obvious case of pairing was observed in this species, and this was unfortunately in a nucleus which had been cut by the microtome knife and showed only eleven chromosomes (Pl. XXXVI, Fig. 62). In this species the pachytene thread passes into the second contraction phase, which is accomplished very rapidly, at a relatively late stage. Hence the thread is much

shortened and thickened, so that it is sometimes possible to trace the course of the pachynema throughout its entire length prior to second contraction (Pl. XXXVI, Figs. 55, 56). When this can be done, the thread is always found to consist of a single continuous strand, which may begin to segment before contraction occurs. This suggests that, when two or more rings occur in 'diakinesis', they have been derived from a single ring during the second contraction phase.

Heterotypic Division.

The fibres of the multipolar spindle are gradually rearranged until the spindle becomes bipolar, the fibres having now extended completely across the cavity. Meanwhile the chromosomes have become very dense and are usually oblong-cylindrical in shape. The rings of chromosomes are drawn towards the equator of the spindle (except in *O. Agari*), where alternate chromosomes of the rings normally become attached to fibres radiating from opposite poles. Whilst the spindle is multipolar the chromosomes simply lie in a mass in the central region, but immediately it becomes bipolar each individual chromosome becomes slightly drawn towards the pole to which it will ultimately pass. They still, however, remain linked together precisely as they were in 'diakinesis'. This gives a somewhat zigzag arrangement to the chromosomes (Pl. XXXV, Figs. 41-7) of any particular circle. A similar arrangement has been described as occurring in several *Oenothera* species (10-15, 22, 33, 46). At this time the rings of chromosomes are apt to become rather contorted, and often when the spindle is examined under the microscope in side view several chromosomes appear to be partly superimposed. Actually the rings are arranged around the periphery of the equatorial region. In *O. novae-scotiae* and *O. eriensis* all fourteen chromosomes are thus arranged, remaining linked together in a continuous ring until the anaphase is somewhat advanced. In *O. ammophila* twelve of the fourteen chromosomes remain linked together as they were in 'diakinesis', assuming the zigzag arrangement, whilst the single pair remains separate and lies at the equator of the spindle (Pl. XXXV, Fig. 47). Unfortunately no material of *O. rubricalyx*, which rendered a study of heterotypic divisions possible, was available. Here we should expect to find the six chromosomes remaining linked together on the heterotypic spindle and the four pairs separating at anaphase in the manner typical of most Angiosperms and of those *Oenothera* species which show pairing. It is hoped that this point may be verified later.

Owing to the contortion of the ring of chromosomes and to the extreme delicacy of the connexions between them, it is very difficult to trace at this stage the entire course of the long continuous zigzag chains formed in *O. novae-scotiae*, *O. eriensis*, and *O. ammophila*; indeed, it is impossible to

do so unless fixation is excellent and the stain correctly differentiated. It is especially difficult in *O. eriensis* owing to the extraordinary length and fineness of the connecting threads between the chromosomes.

In those *Oenothera* species in which pairing of homologues does not regularly take place, no stage which can rightly be termed *heterotypic metaphase* occurs. At no time does a regular grouping of the chromosomes in the equatorial plate normally take place. Immediately the rings cease to lie haphazard in the central region of the multipolar spindle, on the latter becoming bipolar, the individual chromosomes become slightly inclined towards that pole to which they will ultimately pass. The opposing external forces of the spindle-fibres, together with the tensions induced by them in the connecting threads between the chromosomes, cause the latter to lose their oblong-cylindrical form and to become either V- or heart-shaped. As the chromosomes pass to the poles of the spindle, the threads connecting them at first become drawn out. Eventually, however, they break down and are presumably drawn into the substance of the chromosomes (Pl. XXXV, Figs. 49, 50), which gradually assume a more rounded shape. Each diverging group typically arranges itself in a circle of six with one chromosome lying near the centre (Pl. XXXVI, Fig. 73).

This regular passing of adjacent chromosomes of the spireme to opposite poles ensures a regular segregation of chromosomes at each meiotic division. It is not a matter of chance which daughter nucleus any particular chromosome will enter, for some mechanism, as yet undefined, exists ensuring the regular separation of alternating chromosomes of the rings. Nevertheless, irregularities in this zigzag arrangement are sometimes observed in the 'metaphase' or early anaphase stages when two adjacent chromosomes are seen to be passing towards the same pole. This may lead to the daughter nuclei not receiving their proper number of chromosomes. Pl. XXXV, Fig. 44, shows thirteen chromosomes, two adjacent ones being obviously about to pass to the lower pole of the spindle. Another chromosome to the left-hand side of the spindle has become suspended between two chromosomes which are about to pass in opposite directions and must of necessity ultimately go to the same pole as one of them. If this suspended chromosome passes to the upper pole, each daughter nucleus will then receive seven chromosomes. If, however, it passes to the lower pole, one daughter nucleus will receive six chromosomes whilst the other receives eight. Such an uneven distribution (23) does occur fairly frequently and does not apparently interfere with the development of the pollen mother-cell. These six-eight distributions can often be traced into the homotypic metaphase (Pl. XXV, Fig. 54) and anaphase. Their further development has not been traced, but it is quite certain that pollen grains are formed from such pollen mother-cells. When a pollen grain containing eight chromosomes fertilizes a normal egg it would produce a fifteen-chromosome mutant. Those with

six chromosomes presumably do not function in fertilization, as no thirteen-chromosome forms are known.

Sometimes following a six-eight segregation in *O. novae-scotiae* a single chromosome is not included in either telophase group, but is left out in the cytoplasm. As the two groups of chromosomes separate, the extra one may become drawn out into a long narrow thread between them (Pl. XXXV, Fig. 53). Presumably it disintegrates later and is absorbed into the cytoplasm. A similar phenomenon has been described in *O. lata* (30); seven chromosomes having passed into each daughter nucleus, the fifteenth may sometimes be left in the cytoplasm, where it later disintegrates. In *O. Lamarckiana* anaphasic chromosomes may leave a trail of chromatin behind them or may be left outside the daughter nuclei (53).

Six-eight distributions do not always result from irregularities in segregation at anaphase (Pl. XXXV, Fig. 47), as in some cases two sets of adjacent chromosomes are apparently passing to opposite poles. Occasionally the results of five-nine distributions have been observed in heterotypic telophase and later stages. This would presumably arise when four sets of adjacent chromosomes pass towards the same pole.

It was not possible to count easily a sufficient number of cells in which an unbroken ring could be seen at 'metaphase', so as to find the percentage of irregular segregations. An estimate of the proportion of six-eight divisions was, however, made by counting several hundreds of cells in heterotypic telophase and during the homotypic divisions. The results obtained were as follows:

O. novae-scotiae, 4 per cent.

O. eriensis, 7 per cent.

O. ammophila, 6 per cent.

The proportion of irregular segregations would, of course, be considerably greater than this, as irregularities in anaphase do not necessarily result in six-eight distributions.

O. Agari differs from all other species described in that this mechanism, ensuring in most cases a regular segregation of alternating chromosomes of the spireme, does not appear to act. The chromosome connexions seem to break down rather earlier in this species than in other forms, having usually disappeared long before the anaphase is reached. In many cases it is possible to judge the original positions of the chromosomes by their present alinement. When this is done a few cases are found indicating that something approaching the regular zigzag arrangement so characteristic of other forms occurs occasionally in this species (Pl. XXXVI, Figs. 66-72), but of these instances none are entirely devoid of irregularities. In Pl. XXXVI, Fig. 67, the arrangement is similar to that shown by other species, but nevertheless two chromosomes are suspended and each must perforce pass the

same way as one of its neighbours. Pl. XXXVI, Fig. 68, shows a suspended chromosome, and in Pl. XXXVI, Fig. 71, two adjacent chromosomes are going to the same pole. In many anaphase figures some chromosomes take the form of thickened V's as in other species (Pl. XXXVI, Figs. 66-72); these would then appear to have assumed this form owing to the opposing forces of the fibres and the adjacent chromosomes, as occurs in other species. In these same figures, however, chromosomes of rounded and sub-globular shapes also occur.

Despite the fact that no apparent mechanism exists ensuring the regular segregation of alternating chromosomes of the circles formed in prophase, no cases of irregular numbers of chromosomes being received by the daughter nuclei were observed.

The significance of the apparently mechanical segregation of univalents at each reduction division in the pollen mother-cell and of the irregularities which sometimes occur will be discussed later.

In all species by the time the chromosomes reach the poles they have usually assumed an elliptical form. During early telophase a longitudinal split may appear in each chromosome. A clear area appears in the cytoplasm around the chromosomes and a nuclear membrane is deposited (Pl. XXXV, Fig. 51). The nucleus so formed enlarges rapidly, the chromosomes lying at the periphery. The chromosomes themselves enlarge and take often the form of irregular Maltese crosses. Actually the chromosomes elongate and the split halves separate at the ends, remaining, however, in intimate contact in the central region. Occasionally, some of the chromosomes anastomose. A few small nucleoli appear in each daughter nucleus, always in contact with the chromosomes (Pl. XXXV, Fig. 52). The reorganization of the daughter nuclei is never completed. The nucleoli disappear, the chromosomes again contract, the nuclear membrane dissolves as the multipolar homotypic spindle appears. This is often formed before the chromosomes have assumed their former rounded shape, as they are sometimes seen to be still anastomosed. The spindle becomes bipolar and the half-chromosomes rapidly pass to opposite poles. Four grand-daughter nuclei are reorganized as cytokinesis occurs, dividing the pollen mother-cell into four young pollen grains.

DISCUSSION.

The Nucleolus: its Relation to the Chromosomes.

For many years the nucleolus has been under discussion, numerous and varied theories having been put forward as to its constitution and function. Many workers both on plant and animal material have suggested that its function is in some way related to the formation of the chromosomes. Definite organic connexions between the nucleolus and the chromatin thread in prophase have been described in numerous instances, e.g. *Lilium*

Martagon (50), *Galtonia candicans* (20), *Matthiola incana* (2), and *Tmesipteris tannensis* (58). Van Camp (55) describes the threads near the nucleolus in somatic nuclei as being especially thick and then tapering; the nucleolus is probably contributing material to the formation of the chromosomes. Nichols (45) thinks the formation of chromatin to be intimately connected with nucleolar activity in *Sarracenia*, but possibly 'the function of the nucleolus is not so definite as that of the chromosomes and centrosomes'. In this plant globules of material elaborated in the nucleolus escape into the nuclear sap and are absorbed by the linin and distributed along its threads. Dobell (21) holds that in certain *Amoebae* only the karyosome contributes to chromosome formation and consists of granules of chromatin embedded in a plastin matrix. Wager (57) finds that in *Phaseolus* root-tips the nucleolus is concerned in the formation of chromosomes, nucleolar material being transferred to the thread. The nucleolus of the pollen mother-cell of *Ranunculus acris* (54) gives off granules of material which may form a beaded thread. In *Asterias Forbesii* (35) the nucleolus appears to be a storehouse of nutritive material and is connected to the chromatin thread. The same author (34) finds that an extreme case occurs in *Echinaster crassispina*, where the chromosomes are derived solely by the fragmentation of the nucleolus. Ludford (40) finds that in the mollusc *Patella* a single original nucleolus gives rise to two, one oxyphil and the other basophil; the latter is concerned in the formation of chromosomes. Similarly, Baranov (3) has found that in *Galtonia candicans* two nucleoli fuse to give a single large nucleolus, which then breaks into two parts: one, pale staining and spherical; the other, dark staining and lenticular. Various changes occur in the latter, and ultimately in diakinesis five or six smaller spherical nucleoli are found. A bivalent lies in intimate contact with one of the latter, the place of contact staining very deeply, and appearing as one, two, or three dark spots. These may be satellites, or, as the author suggests, it may be that the place of contact stains more deeply with haematoxylin than does the rest of the nucleolus owing to the iron-alum being able to extract less stain. Cardiff (6) holds the view that in *Acer platanoides* and *Claytonia virginica* material flows from the nucleolus into the chromatin thread. At the point of contact of the thread and the nucleolus the latter bulges out into a papillose projection which in some cases looked like a small vacuole escaping.

It has recently been found that in *Lathyrus odoratus* (37) a definite dark-staining body, which may take the form of a papilla, is always present in the nucleolus during the prophase of the heterotypic division of the pollen mother-cell. To this body a single loop of the thread is always attached. The condition found in *Oenothera* is comparable with this. Threads connecting the nucleolus with the spireme have previously been described in the pollen mother-cells of this genus (4, 10, 12, 14) and in the

megaspore mother-cells (17). Cleland (15) has also described an endonucleolus which in some cases is intimately connected with the thread. It now seems definitely established that the chromatin thread is always attached to this endonucleolus during the prophase. This, together with the decrease in staining reaction of the nucleolus during prophase and the corresponding increase in that of the erstwhile reticulum, seems to indicate that chromatin material flows from the nucleolus into the thread by way of the loop or loops attached to the endonucleolus. It is improbable that a definite part of the thread is always connected to the endonucleolus, as quite often more than one loop is attached, or a second loop may be in connexion with a second smaller endonucleolus which is present in either the same or in a different nucleolus. It also appears improbable that the thread moves round, each part in turn coming into intimate contact with the endonucleolus, as sometimes very much thickened threads are found to emanate from the endonucleolus. It would seem that all the chromatin material flows from the nucleolus through the same loop of the chromatin thread, whence it is distributed over the remainder of the spireme. When much-thickened loops are formed the chromatin material is probably flowing from the nucleolus much more quickly than it can be dealt with. This is also a possible explanation of the portions of thread which appear to contain large aggregations of chromatin in *O. novae-scotiae*. Probably material has flowed from the nucleolus more rapidly than it can progress along the thread, hence those parts of the spireme which are relatively close to the endonucleolus become clogged with chromatin material.

'The origin of the endonucleolus is at present unknown. In *Lathyrus odoratus* the nucleolar body is formed from a single surviving portion of the crystal body which is present in the resting stage and later fragments. This is clearly not the case in *Oenothera*, for the presence of the crystalloid is not a constant feature of the resting nucleus throughout the whole of the genus. When a large crystalloid is present in the resting stage, numerous smaller ones are formed from it, but these all disappear before the reticulum gives place to the spireme and before the endonucleolus is visible. The latter appears to be a centre for the elaboration of material which is passed from the nucleolus to the chromatin thread, and consequently retains its intense staining reaction, whilst the nucleolus itself, presumably because it is giving up its reserve material, gradually loses its affinity for chromatin dyes. During early synapsis it is possible to see the endonucleolus only after considerably destaining. It is just possible that this nucleolar inclusion is present in the earliest prophase stages, but is invisible owing to its reaction to chromatin dyes being the same as that of the nucleolus containing it.

As regards the ultimate fate of the endonucleolus in *Oenothera*, little is at present known. During second contraction it is undoubtedly present, and the spireme is attached to it. However, immediately the spireme breaks

away from the nucleolus on emerging from the second contraction knot, the endonucleolus is no longer visible. During 'diakinesis', the nucleolus persists as a pale-staining body, but does not usually become vacuolate. It has been suggested that in the early stages 'prochromatin' is contained in the nucleolus and later gives true chromatin (37), the pale-staining body seen in diakinesis being merely the plastin matrix. The same author has also suggested that material may flow from the cytoplasm through the nucleolus, which during the prophase in *Lathyrus odoratus* is very closely apposed to the nuclear membrane, into the nucleolar body, where it is probably further elaborated, and thence to the chromatin thread. This again is unlikely to occur in *Oenothera*, as, although the nucleolus usually lies against the nuclear membrane, it does occasionally remain more or less spherical and continue to occupy the centre of the cavity.

The pale-staining nucleolus seen in 'diakinesis' disappears quite suddenly, without previously becoming vacuolate or fragmenting, at the same time as the nuclear membrane is dissolved. After this stage, no indication of the presence of a nucleolus is seen until the reorganization of the daughter nuclei in interkinesis, when several small spherical nucleoli arise, usually in contact with the chromosomes.

These observations give support to the view that it is the link framework of the chromosomes rather than the chromatin which is of importance in the continuity of structure from one nuclear generation to the next.

The Linkage of Chromosomes.

The formation of large rings of chromosomes in the later prophase stages, instead of the pairing of homologues to form bivalents, has been described for a number of *Oenothera* species, each species having its own characteristic arrangement which persists throughout the stages corresponding to diakinesis. Occasionally complete pairing occurs, but more often some or all of the chromosomes are arranged in large rings. All conditions from complete pairing to its total absence have been observed.

When some or all of the chromosomes are arranged in rings, and at anaphase alternate chromosomes pass to opposite poles, it is quite clear that they are not arranged according to chance. Taking the case of *O. novae-scotiae* or *O. eriensis*, where all fourteen chromosomes are arranged in a single ring :

The total number of ways of arranging fourteen chromosomes, according to chance, within a single ring $= \underline{13}$.

The number of ways of arranging fourteen chromosomes within a ring so that when alternating members pass to opposite poles no two homologues will go to the same pole $= \frac{6 \cdot 7 \cdot 2^7}{2}$

∴ If the chromosomes are arranged haphazard within the ring, the chances that no non-disjunction will occur

$$\begin{aligned} &= \frac{13}{6} \cdot \frac{7}{7} \cdot 2^6 \\ &= 429:16 \end{aligned}$$

i. e. If the fourteen chromosomes were arranged within the ring according to chance, in a little over 3.5 per cent. of the heterotypic divisions all homologues would go to opposite poles. Thus, a large amount of non-functional pollen would be produced and a number of mutants would be thrown in each generation. Hence it follows that the arrangement cannot be one of chance.¹

As the adjacent chromosomes normally pass to opposite poles at anaphase, it is obvious that if a certain chromosome occupies a particular position in the ring, its mate must lie either next to it or next but two, four, or six. The arrangement of the concatenated chromosomes of the rings in 'diakinesis' is essentially the same as it is on the early spireme before the individual chromosomes are differentiated. In those cases in which a pair is cut off from the spireme prior to 'diakinesis', a single length of thread is cut off and later gives rise to a bivalent. In this case homologues must lie adjacent on the spireme, as they do in other genera when the arrangement is telosynaptic. It is reasonable to suppose that homologues are also adjacent when large rings persist into 'diakinesis'; chromosomes of maternal and paternal origin would probably alternate. It seems to be more than likely that each chromosome has a definite position on the spireme, which it always assumes, the arrangement quite possibly persisting throughout the nuclei of the somatic tissues. As Cleland (14) points out, in such a case each chromosome would be to the whole linkage system as a chromosome is to a chromosome.

In those forms in which pairing and linkage of chromosomes regularly occur, the inheritance of the characters borne by the pairs will take place probably in the normal Mendelian manner. The joining of the chromosomes into large rings and the ultimate segregation of those chromosomes which alternate is essentially a new form of linkage, each linkage group acting as a unit in heredity. When complete pairing occurs and linkage is observed between certain morphological characters, the factors responsible for these characters must be contained within a single chromosome. However, when the manner of segregation at anaphase is controlled by the linkage of a number of chromosomes, the factors responsible for linked morphological characters need not be situated in the same chromosome, but must be in the same group of linked chromosomes. Absence of pairing of chromosomes is obviously due to incompatibility between homologues,

¹ These mathematical results differ from those obtained by Cleland (15) for the case of *O. muricata*, but lead to the same general conclusions.

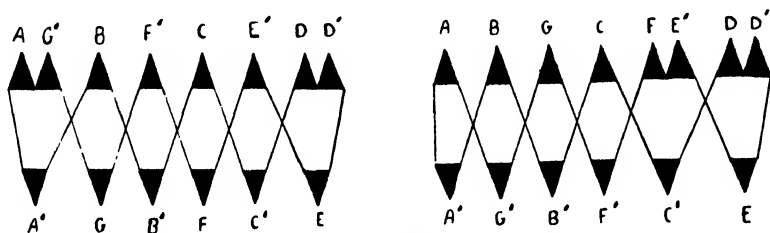
the attraction between the telosynaptically arranged chromosomes being as great as that existing between the homologues.

Irregularities. In a few exceptional cases departures from the normal constitution of the rings are observed; these may be of little or of considerable importance genetically. In *O. ammophila* a continuous chain of fourteen chromosomes was found in one case to replace the normal arrangement of twelve chromosomes in a ring and a bivalent. When anaphase is reached in this case, two linkage groups each of seven univalents will pass to opposite poles, whilst normally two linkage groups each of six chromosomes segregate, the remaining bivalent assorting quite independently. Thus in fifty per cent. of the divisions a complex precisely similar to that received by the daughter nuclei in the abnormal case quoted passes to the pole; in the remaining divisions the complex received differs by one chromosome. Thus, such an abnormality would not result in any new chromosome complex, but would simply alter the proportion in which the normal complexes occur. If, however, an additional pair were cut off from the main ring, genetical aberrations might occur. Such a case was found in *O. rubricalyx*, where a ring of four chromosomes and five bivalents replaced the normal ring of six with four pairs. Here the unusual cytological condition produced in prophase may result in the formation of new complexes depending on which way the members of the abnormally free pair pass at anaphase. Thus, a cytological abnormality, where rather more than the normal amount of chromosome linkage occurs, may result in the different kinds of pollen being produced in unusual proportions. However, when the amount of chromosome linkage is reduced by the breaking of the rings usually formed, entirely new chromosome complexes may be received by the pollen grains and might conceivably result in the production of aberrant forms. Such irregularities in the constitution of the spireme are observed only very occasionally.

Abnormalities in the segregation of the linked chromosomes at anaphase are of considerably more frequent occurrence, and are probably much more important genetically. These abnormalities arise through two adjacent chromosomes of the spireme passing to the same daughter nucleus. This may result in a six-eight distribution. If, however, the irregularity is to some extent compensated by two other adjacent chromosomes passing to the opposite pole, the normal number of chromosomes will go to each pole, but probably in each daughter nucleus one univalent will be missing whilst another is duplicated. It is just possible that an abnormality occurred in the order of the chromosomes on the spireme, and that this irregularity is being rectified, so giving the erroneous idea that an abnormality is occurring in anaphase. If such is the case, each daughter nucleus and ultimately each pollen grain would receive its normal complement of chromosomes. This, however, could not account for the six-eight distribu-

tions which occur, and there are several further possibilities as to the significance of these irregularities.

Irregularities in segregation may be the result of the non-disjunction of a pair of chromosomes, the daughter nuclei thus receiving different numbers of chromosomes (Text-fig. 1). Such segregation also results in the exchange of homologues between the two daughter nuclei. Usually, six chromosomes pass to one pole whilst eight go to the other; occasionally, however, five–nine divisions are observed, and presumably they arose in a similar manner. The daughter nuclei formed as a result of such an uneven segregation at meiotic division continue their development in the normal way.



TEXT-FIG. 1. Diagrams illustrating six-eight distribution of chromosomes.

Undoubtedly, pollen grains with eight chromosomes are formed and function in fertilization with the ultimate production of trisomic mutations. Presumably those with six chromosomes do not function, as no thirteen chromosome mutants are known. In *O. novae-scotiae* it is possible that the grains with eight chromosomes do not function. It is known that in this species an irregularity in meiotic segregation is often rectified later by the omission of the extra chromosome from the daughter nucleus.

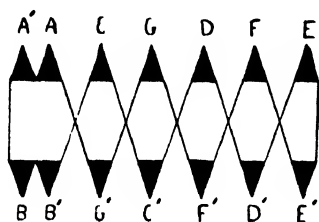
Those irregular segregations which do not result in an uneven distribution of chromosomes may still be of considerable importance genetically. Double non-disjunction may occur, homologues passing towards the same pole in each of two cases. The diagram (Text-fig. 2) illustrates a case of double non-disjunction in a form where all fourteen chromosomes remain linked.

Chromosomes B and A' are exchanged between the two complexes. Thus one daughter nucleus will receive both homologues B and B' but no A' chromosome, whilst the other receives A and A' but no B. Such an abnormality might result in sterility or in the production of aberrant forms.

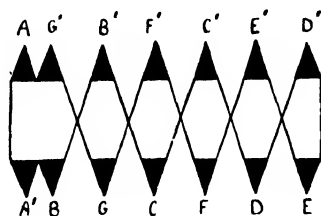
By a continuous series of breeding experiments Renner (49) has found that definite complexes are received by the daughter nuclei after each reduction division. He has been able to analyse the gene complexes into their component Mendelian units. Occasionally, however, exchange of material takes place between the complexes normally formed. The irregularities observed afford a cytological explanation of this occurrence, a cytological basis having been found for a process comparable with and

having the same ultimate effect as 'crossing over', although larger numbers of characters are probably involved. It is in fact the interchange of whole chromosomes between the two complexes.

This is illustrated diagrammatically by Text-fig. 3. Actually the irregularity looks precisely similar to that illustrated in Text-fig. 2, but the significance is different. This case results in the interchange of homologues between the two daughter nuclei. In Text-fig. 3 the chromosomes A and A' are interchanged. Such an irregularity would result in aberrations or, if sufficient characters are involved, in mutations.



TEXT-FIG. 2. Diagram illustrating double non-disjunction.



TEXT-FIG. 3. Diagram illustrating exchange of chromosomes between the two complexes.

As previously stated, it was not possible to estimate accurately the total proportions of irregularities, but they are obviously considerably greater than the percentage of six-eight distributions which were determined (page 795). In connexion with these irregularities, which are doubtless in part responsible for the production of non-functional gametes, it is of interest to note the proportions of sterile pollen produced by the different species. These counts were made at the end of the flowering season and consequently should indicate a maximum. They were found to be:

O. novae-scotiae, 29 per cent.; *O. eriensis*, 53 per cent.; *O. rubricalyx*, 8.6 per cent.

The proportion of seeds with abortive embryos was also determined:

O. novae-scotiae, 7 per cent.; *O. eriensis*, 11 per cent.; *O. rubricalyx*, 25 per cent.

The significance of these results cannot be properly interpreted until more is known of the process of meiosis in the megaspore mother-cell. It is not at present known whether the mode of meiosis on the female side is precisely the same as that which occurs in the pollen mother-cells. Davis (18) has described strings of linked chromosomes in the megaspore mother-cells of *O. biennis*, so it seems likely that regular groups of linked chromosomes are formed here as they are in the corresponding pollen mother-cell; in which case we should expect occasionally to encounter similar abnormal segregations. Presumably these are not so frequent in the megaspore mother-cell as in the pollen mother-cell.

In contrast to most other species, no regularity of chromosome configuration in 'diakinesis' or of segregation at anaphase is observed in *O. Agari*. Large rings of chromosomes are formed in 'diakinesis', but these are not always of the same constitution. When large rings are formed we expect to find homologues included within the same ring. This appears to be the case in all species described, except *O. Agari*. Here, rings containing odd numbers of chromosomes are frequently formed; obviously certain of the homologues must then be separated. The chromosomes of *O. Agari* exhibit irregular behaviour also on the heterotypic spindle. In these stages practically no indication is found of the zigzag arrangement so strikingly characteristic of most species. The chromosomes come to the equator of the spindle in a very irregular manner, and segregation is apparently entirely a matter of chance. When circles containing odd numbers of chromosomes are found, the mechanism which in other species ensures the separation of homologues at anaphase cannot conceivably function. It is remarkable that in this species it was impossible to find any instances of non-disjunction, and the percentage of sterile pollen produced is not extraordinarily high. Counts were made at the end of the flowering season in October, and would hence be maximum. The proportion was found to be about 26 per cent., and that of non-viable seeds produced was 9 per cent. *O. Agari* does not fall into the same group as the other species examined and is sterile with them. However, it has a peculiarly characteristic habit and, despite its extraordinary cytological behaviour during the three years in which it has been under observation, with the exception of one mutant which appeared in 1925, it has been of extremely uniform appearance and behaviour.

General Genetical Considerations.

Tables indicating the characteristic arrangement of the chromosomes in 'diakinesis' in the different species investigated by various workers are appended.

The linking together of chromosomes, which appears to be general throughout the genus *Oenothera*, is by no means a common phenomenon among other Angiosperms. Indications of such linkage have, however, recently been described in related genera. For example, in *Eucharidium concinnum* (52) seven gemini are usually observed in 'diakinesis'. Not rarely, however, two pairs remain linked together, forming a chain of four chromosomes which persists on to the spindle. A similar tendency of the telosynaptically arranged chromosomes to remain linked in 'diakinesis' has been observed in a hybrid *Godetia* (32). Also Osawa (47) found that the chromosomes of *Daphne odora* tended not to pair, but to remain linked in longer or shorter chains which persisted on to the heterotypic spindle.

Various theories as to the cause and the significance of the formation

of such groups of linked chromosomes have been put forward. When Cleland (10) first found that the configuration observed in 'diakinesis' in

TABLE I.

<i>Species.</i>	<i>Haploid Number of Chromosomes.</i>	<i>Chromosome Arrangement in 'Diakinesis'.</i>	<i>Author.</i>
<i>O. grandiflora</i> , Ser.	7	7 pairs	Davis, 1909
<i>O. Hookeri</i>	7	7 pairs	Schwemmle, 1924
<i>O. franciscana</i>	7	5 pairs and ring of 4	Cleland, 1922
<i>O. Lamarckiana</i>	7	1 pair and ring of 12	Cleland, 1925
"	7	7 pairs	Håkansson, 1926
<i>O. ammiophila</i> , Focke	7	1 pair and ring of 12	Boedijn, 1924
<i>O. suaveolens</i>	7	12-14 linked	Sheffield, 1927
<i>O. Cockerelli</i>	7	12-14 linked	Oehlkers, 1926
<i>O. strigosa</i>	7	12-14 linked	" 1926
<i>O. muricata</i>	7	Ring of 14	" 1926
<i>O. novae-scotiae</i>	7	Ring of 14	Cleland, 1925
<i>O. eriensis</i>	7	Ring of 14	Sheffield, 1927
<i>O. biennis</i>	7	2 rings of 6 and 8 respectively	" 1927
<i>O. biennis sulfurea</i>			Cleland, 1923
<i>O. Agari</i>	7	Closed rings are formed, but these show no constancy in their constitution	" 1926
			Emerson, 1924
			Sheffield, 1927

TABLE II.

<i>Mutants of O. Lamarckiana.</i>	<i>Haploid Number of Chromosomes.</i>	<i>Chromosome Arrangement in 'Diakinesis'.</i>	<i>Author.</i>
<i>O. blandina</i>	7	7 pairs	Cleland, 1925
<i>O. deserens</i>	7	7 pairs	" 1925
<i>O. oblonga</i>	15/2	5 pairs and ring of 5	" 1923
<i>O. rubrinervis</i>	7	4 pairs and ring of 6 (?)	" 1925
<i>O. rubricalyx</i>	7	4 pairs and ring of 6	Sheffield, 1927
¹ <i>O. rubricalyx</i> 'After-glow'	7	3 pairs and ring of 8	Cleland, 1925
<i>O. rubrisepala</i>	7	4 pairs and ring of 6	Håkansson, 1926
<i>O. planifolia</i>	7	1 pair and ring of 12	" 1926
<i>O. flavescens</i>	7	1 pair and ring of 12	" 1926
<i>O. dependens</i>	15/2	1 pair and ring of 13 (ring often broken)	" 1926
<i>O. dentata</i>	15/2	1 pair, others may be linked	" 1926
<i>O. obscura</i>	15/2	1 pair and ring of 13 or irregular	" 1926
<i>O. gigantea</i> diploid	7	1 pair and ring of 12	" 1926
<i>O. curta</i>	15/2	Configuration unknown	" 1926
<i>O. excelsa</i>	21/2	Trivalent often seen: other chromosomes may be connected	" 1926
<i>O. gigantea</i>	14	2 pairs or ring of 4	" 1926

O. franciscana was constant throughout the pollen mother-cells of that species, he emphasized the apparent correlation existing between hereditary

¹ See p. 807.

instability and the irregular behaviour of the chromosomes in reduction division on the one hand, and between stability and the regular uniform behaviour on the other. In support of this view he quoted the cases of *O. grandiflora*, where 100 per cent. pairing is constantly observed (17), and *O. franciscana*, where five pairs regularly occur, the other four chromosomes forming a ring. He laid great stress on the purity and stability of the latter species. In 1916, however, the same species was studied by Gates (27) growing in large wild colonies near San Francisco, when it was found that wild variations were conspicuous in the size of the flower, the pigmentation, and the number of red papillae on the sepals. It was evidently a population of interbreeding forms, and must have been heterozygous for a number of characters.

TABLE III.

<i>Hybrids.</i>	<i>Haploid Number of Chromo- somes.</i>	<i>Chromosome Arrange- ment in 'Diakinesis'.</i>	<i>Author.</i>
<i>O. (suaveolens</i> × <i>strigosa</i>) <i>flava</i>	7	7 pairs	Oehlkers, 1926
<i>O. Hewetti</i> × <i>rubricalyx</i>	15/2	1-5 pairs and rest linked	Gates, 1923
<i>O. franciscana sulfurea</i>	7	1 pair and ring of 12	Cleland, 1924
<i>O. (suaveolens</i> × <i>strigosa</i>) <i>albata</i>	7	1 pair and ring of 12	Oehlkers, 1926
<i>O. Lamarckiana</i> × <i>bi- ennis</i>	7	1 pair and ring of 12	Håkansson, 1926

Later, a cytological examination of several other species, some of which were of known hybrid origin, led Cleland (12) to support the suggestion previously made (23), to the effect that the failure of homologues to pair in certain species might be due to hybridity. The lack of pairing seemed to be correlated with sterility; in *O. franciscana* there is little seed or pollen sterility, whilst in *O. franciscana sulfurea*, where only one bivalent is cut off from the main spireme, 50 per cent. of the pollen aborts.

Håkansson (32) has made a study of several species of *Godetia*, and finds that in all of them the gemini form ring pairs in 'diakinesis'. Nevertheless, in a hybrid form, *G. amoena* × *Whitneyi*, although the evidence is incomplete, it suggests a general lack of pairing, the chromosomes remaining attached end to end. This author consequently puts forward the view that the chromosome linkage in *Oenothera* arose originally through hybridization. Later (33), he examined several derivatives of *O. Lamarckiana* and found in them a great diversity of chromosome configuration. However, those forms in which little pairing occurs show a high percentage of pollen sterility, whilst those where much pairing occurs show little sterility. Although the pollen mother-cell nuclei of some of the forms described in this paper are totally devoid of pairing, they do not all show a large proportion of pollen sterility. In *O. eriensis* the percentage of abortive pollen

is fairly high, but in *O. novae-scotiae*, where all the chromosomes remain joined together in a ring, the proportion of sterile pollen is less than 30 per cent.; in *O. Agari* where practically no pairing is observed, the proportion is about 26 per cent. In *O. rubricalyx*, however, some pairing of homologues does occur, and here the pollen sterility is considerably less.

A similar linkage of chromosomes resulting in a very irregular metaphase occurs in *Daphne odora* (47), and appears to be correlated with much sterility. It is suggested, however, that these irregularities arose through long cultivation or by mutation.

It may possibly be more difficult for telosynaptically arranged chromosomes to pair than for parasynaptically arranged ones. Pairing, however, is accomplished in a number of such forms, hence the failure of the chromosomes to pair must be due, at least in part, to lack of affinity between the homologues. It has been suggested that the chromosomes which pair are relatively homozygous, whilst those remaining linked are relatively heterozygous, the incompatibility between the latter not having necessarily arisen through hybrid origin, but rather through the gradual accumulation of gene mutations within the chromosomes, a process which has probably been aided by balanced lethal factors.

In this connexion it is of interest to note the difference in the results of the cytological study of *O. rubricalyx* described in this paper and those described by Cleland (13). *O. rubricalyx* originated as a mutant in a pure culture of *O. rubrinervis* in 1907 (24). The offspring of this plant split into two distinct groups, *rubrinervis* and *rubricalyx*. In the third generation of selfed plants from the original mutant, a pure race of plants was obtained, the female parent having been homozygous for red. The mutation has been continued in a pure line, and cytological material from this original stock was examined. Morphologically, *O. rubricalyx* is identical with *O. rubrinervis*, differing from it only in pigmentation. The outstanding cytological features of *O. rubricalyx* as described in this paper are identical with those put forward tentatively for *O. rubrinervis* by Cleland (13). The material studied as *O. rubricalyx* by Cleland (13) was in reality Messrs. Sutton's 'Afterglow', which was derived from seed acquired from Professor Gates. This seed was obtained from open pollinated flowers, and had obviously been crossed with *O. grandiflora* (24). Although a *rubricalyx*-like form had segregated out again, the cytology had apparently been affected, the amount of pairing between homologues having been reduced.

It has been suggested that a parallel exists between the heterozygous character and the formation of linkage groups, and between the homozygous nature of a species and the pairing of homologous chromosomes prior to reduction division (14). The lack of affinity between pairs of chromosomes which are relatively heterozygous is the probable cause of their remaining linked as they were on the spireme, whilst the greater

attraction existing between the relatively homozygous pairs causes them to mate. If the linkage mechanism causes coupling of such relatively heterozygous chromosomes of similar parental origin, whilst the more homozygous pairs assort in a normal Mendelian manner, the number of differently effective complexes which can be formed at any reduction division will depend solely on the number of groups of linked chromosomes present. E. g. in a species such as *O. Lamarckiana*, where twelve chromosomes form a ring $AA'BB'CC'DD'EE'FF'$, a single ring pair GG' being cut off, the possible chromosome complements which might be received by the daughter nuclei are: (a) $ABCDEFGG$; (b) $A'B'C'D'E'F'G'$; (c) $ABCDEFGG'$; and (d) $A'B'C'D'E'F'G$, according to the position which the pair GG' assumes on the spindle prior to segregation. If, however, G and G' are relatively homozygous, so long as they separate, it is immaterial to which pole either one passes at anaphase, as only two differently effective complexes can be formed, (a) and (c) having the same effect, as also have (b) and (d). Thus in such a case only two different kinds of pollen would be formed. This, according to Renner's analysis (38), is actually the case in *O. Lamarckiana*.

The view that relatively homozygous chromosomes pair, whilst the more heterozygous remain joined together as they were on the spireme, is incompatible with some experimental results. Comparing the cytological behaviour of a form like *O. rubricalyx* with a species like *O. novae-scotiae*, the former shows a considerable amount of pairing, whilst the latter is completely devoid of it. Yet in view of the origin and of the genetical behaviour of the two species, it is difficult to believe that the former is more homozygous than the latter. Further, Gates (27) has described the cytology of a trisomic mutant which appeared in the F_1 generation of the cross *O. rubricalyx* \times *Hewetti*. Here, several ring pairs of chromosomes are often observed in 'diakinesis'. The number of pairs found is not constant, but quite frequently as many as five occur, usually not more than one of them persisting on to the spindle.

The linkage of chromosomes may be an indication of the hybrid origin of a species, but it is certain that all hybrids are not thus characterized; nor does a large proportion of pairing denote purity of origin. This linkage which is known to exist between the chromosomes of certain species does, however, suggest an explanation of the regular behaviour of certain plants of known hybrid origin, the occurrence of the phenomenon enabling certain hybrids to breed true. As the mode of segregation of the chromosomes at anaphase is controlled by the groups of linked chromosomes which are formed in prophase, it is possible for any normal nucleus to pass on to its daughter nuclei at reduction division only complexes of definite constitution. This results in a linkage, not only between the factors of individual chromosomes, but between all the genes of a group of linked chromosomes. Whereas, if in a hybrid all the chromosomes form separate pairs during

prophase, linkage exists only between the factors of single chromosomes, and as many as 2^7 different complexes may be formed from reduction divisions. If less pairing occurs, the possible number of complexes is proportionately reduced until, when linkage is complete, only two different complexes can be derived.

Some hybrid races are known which do breed true, such as *O. muricata* \times *biennis* (56). Numerous instances might be quoted where a cross results in the production of twin hybrids, one of which breeds true, whilst the other splits in subsequent generations. Oehlkers (46), having made a study of the cytological and genetical behaviour of certain *Oenothera* species and crosses, was led to connect the presence and absence of free gemini with the splitting or constancy of the form. *O. suaveolens* was crossed with *O. strigosa*, in both parental types the chromosomes remaining linked in 'diakinesis'. Twin hybrids were produced and were called, according to Renner's terminology, *O. (suaveolens* \times *strigosa)* *flava* and *O. (suaveolens* \times *strigosa)* *albata*. In subsequent generations the former splits into a long series of forms: this hybrid and all its descendants show only free gemini. *O. (suaveolens* \times *strigosa)* *albata*, however, behaves rather more constantly, and in 'diakinesis' shows linked chromosomes. Similarly, if *O. Lamarckiana* or some of its derivatives are crossed as pollen parent with *O. biennis*, Chicago, or *O. cruciata*, twin types *densa* and *laxa* are produced; *densa* remains constant, whilst *laxa* splits off a third type, *atra*. In all probability, if the cytological behaviour of these hybrids were known, it would be found that in *densa* the majority of the univalents remain linked in 'diakinesis', while in *laxa* a larger proportion of pairing occurs. If, however, *O. Lamarckiana* or its derivatives are crossed either way with *Cockerelli*, *Hookeri*, or *strigosa*, or as pollen parent with *biennis* or *muricata*, or as seed parent with *biennis*, Chicago, twin hybrids are again produced, but in all cases both of these breed true in later generations (56). The only feasible explanation forthcoming as to this behaviour is that little or no pairing occurs in prophase in these hybrids, the majority of the chromosomes remaining linked.

Until it is known whether or not the behaviour of the chromosomes at reduction division in the embryo-sac mother-cell is precisely the same as that in the corresponding pollen mother-cell, it is impossible to correlate successfully the genetical behaviour of a species with its cytological peculiarities. When this is known and series of crosses between various species are made, a cytological study of the parental types and of the hybrids produced should throw some light on the reason for the linkage of certain chromosomes and on the nature of the pairing within the genus. It is hoped that it will soon be possible to carry out such investigations.

SUMMARY.

1. Certain stages in the meiotic divisions occurring in the pollen mother-cells of five different species of *Oenothera* are described: *O. novae-scotiae*, *O. eriensis*, *O. rubricalyx*, *O. ammophila*, and *O. Agari*. In each of these forms the diploid number of chromosomes is fourteen.

2. The resting and early prophase nuclei are very similar in all these species.

When the nucleus is in the resting stage prior to meiotic division, a granular reticulum lies towards the periphery of the nuclear cavity, the centre of which is occupied by a large spherical nucleolus. The nucleolus may contain a vacuole which, when present, often includes a crystalloid.

3. The reticulum becomes simplified. The crystalloid, when present within the nucleolus, gives off a succession of small fragments, each lying within its own vacuole, the nucleolus thus becoming honeycombed. The nucleolus may bud.

4. The small crystals and the vacuoles containing them disappear. The nucleolus passes towards the periphery of the cavity and usually becomes flattened against the membrane. The reticulum gives place to a long, fine, continuous spireme, which is attached to an inclusion within the nucleolus—the endonucleolus.

5. The thread becomes drawn into the synizetic knot, synizesis lasting for a considerable time.

6. The synizetic knot unfolds, revealing a rather coarser thread. During the open spireme stage the pachynema continues to shorten and thicken.

7. The thread undergoes a second contraction, the time of occurrence of this phase, and the period occupied by it, varying in different species. Segmentation of the spireme takes place about this time.

8. On emerging from the second contraction knot, the thread breaks away from the nucleolar inclusion, to which it has remained attached throughout the prophase. The endonucleolus disappears.

9. During 'diakinesis' the chromosomes are not usually paired, but remain attached together in long closed chains. The configuration of the chromosomes is fairly constant in most species at this time. In *O. novae-scotiae* and *O. eriensis* the chromosomes are arranged in a single ring of fourteen univalents; in *O. ammophila* one pair is cut off from the rest, which lie in a continuous chain; while in *O. rubricalyx* four pairs become cut off, leaving a ring of six. The chromosomes of *O. Agari* form closed rings, but these are not constant in their construction. In all cases when several rings are formed they may be linked together.

10. During 'diakinesis' the chromosomes continue to condense. The multipolar spindle is formed and the nuclear membrane is dissolved. The

nucleolus disappears quite suddenly. The chromosome arrangement seen in 'diakinesis' persists on to the multipolar spindle.

11. The spindle becomes bipolar and the chains of chromosomes are drawn towards the equatorial region. Adjacent chromosomes of the rings become attached to fibres emanating from opposite poles, resulting in a zigzag arrangement, which replaces the normal metaphase. Any pairs of chromosomes which have been formed assort independently. This phase is not observed in *O. Agari*. Irregularities are occasionally found in the other species.

12. At anaphase the linked chromosomes become V-shaped and adjacent ones pass to opposite poles, the connexions breaking down. The univalents become more rounded.

13. At telophase the chromosomes are frequently arranged in a ring of six with one in the centre. They may show the homotypic split. A nuclear membrane is formed and the reorganization of the daughter nucleus begins.

14. Interkinesis is passed over rapidly and homotypic divisions occur in the normal manner.

15. The genetical significance of the cytological events described is briefly discussed.

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EXPLANATION OF PLATES XXXIV-XXXVI.

Illustrating Miss Sheffield's paper on Cytological Studies of certain Meiotic Stages in *Oenothera*.

All figures were drawn with a camera lucida and have been reproduced without reduction.

Figs. 1 a, 2 a, and 2 b were drawn under a 2-mm. imm. Zeiss N.A. 1.4 with Zeiss comp. oc. 18. Magnification $\times 3,300$.

Figs. 41-8 were drawn under a 2-mm. imm. Zeiss N.A. 1.4 with Zeiss comp. oc. 12. Magnification $\times 2,500$.

All other figures were drawn under a $\frac{1}{2}$ -in. imm. Swift N.A. 1.25 with Zeiss comp. oc. 12. Magnification $\times 2,500$.

PLATE XXXIV.

Early Prophase.

Fig. 1. *O. amnophila*. A resting nucleus of a pollen mother-cell. The reticulum is fine-meshed and granular. A spherical vacuolate nucleolus occupies the centre of the cavity.

Fig. 1 a. *O. rubricalyx*. A nucleolus containing a polyhedral crystalloid within a vacuole.

Fig. 2. *O. rubricalyx*. Very early prophase. The reticulum has become simplified.

Fig. 2 a. *O. rubricalyx*. A budding nucleolus. The crystalloid has given off a single fragment which is contained within its own vacuole.

Fig. 2 b. *O. rubricalyx*. A nucleolus. The crystalloid has now given off numerous fragments, each lying within its own vacuole.

Fig. 3. *O. eriensis*. The reticulum has given place to a long, fine, continuous spireme, which is attached to the endonucleolus. The nucleolus is passing to the periphery of the nuclear cavity and has become slightly flattened. A second very small nucleolus is present.

Fig. 4. *O. novae-scotiae*. The chromatin thread is being drawn into the synizetic knot. A bi-convex nucleolus is lying against the nuclear membrane.

Fig. 5. *O. eriensis*. Synizesis. A very much thickened loop of the thread is connected to the endonucleolus.

Fig. 6. *O. ammophila*. Synizesis. The nucleolus is peculiarly shaped.

Fig. 7. *O. eriensis*. Synizesis.

Fig. 8. *O. eriensis*. The thread is loosening from the synizetic knot and is considerably thickened. A large irregularly shaped endonucleolus is present in the nucleolus.

Fig. 9. *O. ammophila*. The synizetic knot is unfolding. The thread is thickened and is attached to the endonucleolus.

Figs. 10 and 11. *O. novae-scotiae*. Very early prophase. Several large aggregations of chromatin are enmeshed in the reticulum.

Fig. 12. *O. novae-scotiae*. Synizesis. The large chromatin aggregations still persist.

O. eriensis.

Fig. 13. Open spireme. The chromatin thread is considerably thicker than previously (Fig. 8), and is so much shorter that it can now be traced throughout its entire length. The spireme is beginning to segment.

Fig. 14. The thread has been drawn into a tight second contraction knot.

Fig. 15. The second contraction knot is beginning to loosen, but the thread is still attached to the endonucleolus.

Fig. 16. 'Diakinesis.' The thread has become constricted into fourteen sections, each of which represents a chromosome. The latter are spongy in texture and are connected by threads, some of which are extraordinarily long and fine. The spireme is continuous, but it is too long to lie within the nuclear cavity without becoming looped and contorted.

The nucleolus is always present in 'diakinesis', although the endonucleolus has disappeared. In this and some other figures the nucleolus would tend to obscure the arrangement of the chromosomes, and has therefore not been represented in the drawings.

Figs. 17 and 18. Slightly later than Fig. 16. The chromosomes continue to condense.

Fig. 19. The nuclear membrane is dissolved, and the nucleolus has finally disappeared. The ring of chromosomes has persisted on to the multipolar spindle.

O. novae-scotiae.

Figs. 20 and 21. 'Diakinesis.' Fourteen chromosomes are joined together into a single closed chain.

Figs. 22 and 23. 'Diakinesis'. The chromosomes appear to be scattered. This is probably due to the treatment to which the material has been subjected.

Fig. 24. Abnormal 'diakinesis'. A ring of six chromosomes is present, the remaining eight chromosomes being arranged irregularly.

Figs. 25 and 26. The spireme has persisted on to the multipolar spindle as in Fig. 19.

O. ammophila.

Fig. 27. Second contraction knot. A short length of the spireme has become cut off from the rest and has formed a ring which is linked to the main portion of the thread.

Figs. 28 and 29. Slightly later than Fig. 27. The thread is thicker, and is becoming constricted to form the chromosomes. A single pair has separated from the rest of the spireme.

Figs. 30 and 31. 'Diakinesis.' A ring of twelve chromosomes and a bivalent lie within the nuclear cavity.

Fig. 32. The nuclear membrane and the nucleolus have disappeared. The chromosomes are lying at the centre of the multipolar spindle, arranged precisely as they were in 'diakinesis'.

Fig. 33. The chromosomes are arranged on the multipolar spindle in an unusual manner, all fourteen being joined together in a single ring.

O. rubricalyx.

Fig. 34. Second contraction. Four loops of thread are projecting from a central knot.

Fig. 35. 'Diakinesis.' Four ring pairs of chromosomes have become cut off from the other six univalents, which form a ring. Two of the pairs are linked to the large ring.

Figs. 36-9. Similar to Fig. 35. The ring pairs and the large ring are interlinked in various ways.

Fig. 40. Abnormal 'diakinesis'. Five ring pairs of chromosomes with a ring of four univalents replace the usual configuration.

PLATE XXXV.

Heterotypic Division.

Figs. 41-3. *O. novae-scotiae*. 'Metaphase.' Alternate chromosomes of the ring are about to pass to opposite poles of the bipolar spindle. This results in a zigzag arrangement of the V-shaped chromosomes, which are joined together by fine threads.

Fig. 44. *O. novae-scotiae*. Abnormal 'metaphase'. Two adjacent chromosomes are about to pass to the lower pole. Another chromosome is suspended between two which will shortly pass to opposite poles. (The section shows only thirteen chromosomes.)

Fig. 45. *O. novae-scotiae*. Irregular 'metaphase'. Two adjacent chromosomes are passing to the upper and two to the lower pole of the spindle. (The section shows only twelve chromosomes.)

Fig. 46. *O. eriensis*. Irregular 'metaphase'.

Fig. 47. *O. ammophila*. 'Metaphase.' Twelve chromosomes are still joined as they were in the late prophase. Adjacent chromosomes of the ring are, however, about to pass to opposite poles, thus giving a zigzag arrangement. The bivalent lying to the left side of the spindle assort independently.

Fig. 48. *O. ammophila*. Irregular 'metaphase'. Two adjacent chromosomes are passing to the lower pole.

Fig. 49. *O. novae-scotiae*. Early anaphase. Adjacent chromosomes are drawing apart; most of the connexions between them have broken down.

Fig. 50. *O. novae-scotiae*. Anaphase. The two groups of chromosomes have drawn farther apart. Some of the chromosomes have lost their former shape, being now more rounded. A single connexion between two of them is still persisting.

Fig. 51. *O. novae-scotiae*. Telophase. A membrane has formed around one group of chromosomes. The former position of the spindle is indicated by striations in the cytoplasm. Some of the chromosomes show the homotypic split.

Fig. 52. *O. novae-scotiae*. Interkinesis. The reorganization of the daughter nuclei has commenced. Several small nucleoli are making their appearance.

Fig. 53. *O. novae-scotiae*. Telophase: abnormal condition resulting probably from a six-eight distribution of chromosomes. The left-hand group contains six chromosomes, whilst that on the right contains the normal number. The fourteenth chromosome has remained in the cytoplasm, where it has become pulled out into a long thread.

Fig. 54. *O. novae-scotiae*. Homotypic metaphase: abnormal condition arising from a six-eight distribution of chromosomes in heterotypic division. One metaphase plate contains eight; the other, six chromosomes.

PLATE XXXVI.

O. Agari.

Fig. 55. Open spireme. A single continuous pachytene thread lies within the nuclear cavity.

Fig. 56. Pachynema. Segmentation of the thread is commencing prior to second contraction.

Fig. 57. 'Diakinesis.' Fourteen univalents arranged in a single ring. The chromosomes are very spongy.

Fig. 58. Similar to Fig. 57, but slightly later. The chromosomes are much condensed.

Fig. 59. 'Diakinesis.' The chromosomes are arranged in four closed rings, two of which each contain three chromosomes.

Fig. 60. 'Diakinesis.' The nucleus is cut, only twelve chromosomes being included in this section. Eight of these are arranged in one ring and three in another.

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Fig. 61. The chromosomes are lying on the multipolar spindle in three distinct focal planes and appear to have been arranged in three rings consisting of four, five, and five chromosomes respectively.

Fig. 61 *b*. Analysis of Fig. 61.

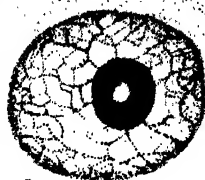
Fig. 62. 'Metaphase.' As the nucleus was cut by the microtome knife only eleven chromosomes are included in this section. These are arranged in circles, a ring pair being visible in bottom focus.

Figs. 63-70. 'Metaphase.' In most of these figures the chromosomes are arranged very irregularly, and in no way resemble Figs. 41-8.

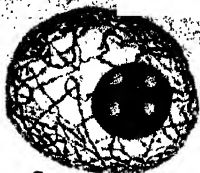
Fig. 71. Anaphase. Two joined chromosomes seem to be passing to the upper pole. The chromosomes are not regularly arranged as in Fig. 49.

Fig. 72. Anaphase. Like Fig. 71, this figure is irregular. A few of the chromosomes are V-shaped, but the majority are rounded.

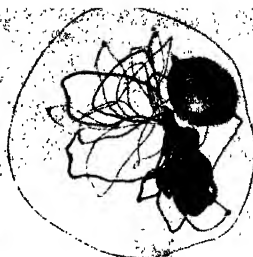
Fig. 73. Anaphase. In polar view each group of chromosomes is arranged roughly in a ring of six, with a seventh lying near the centre. This arrangement in anaphase is typical of many species.



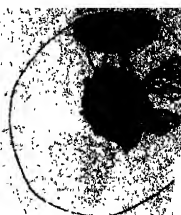
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1a.



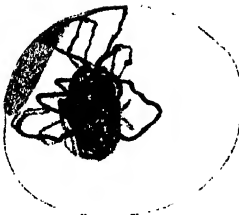
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11.



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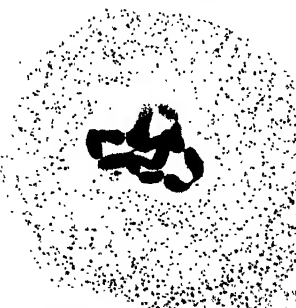
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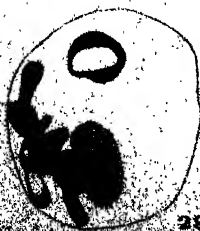
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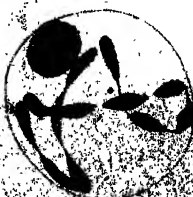
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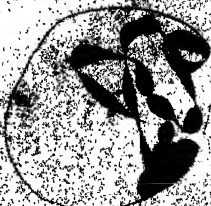
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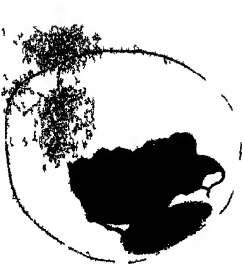
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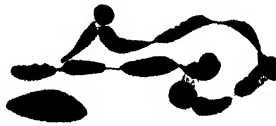
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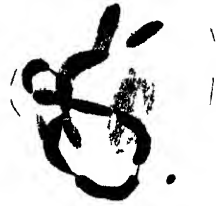
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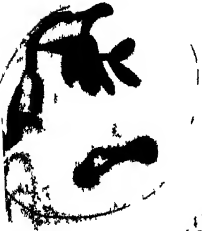
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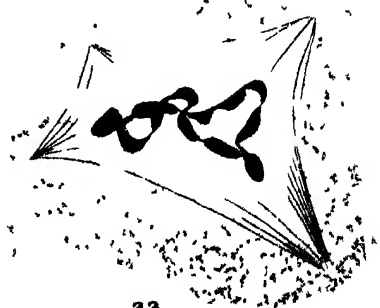
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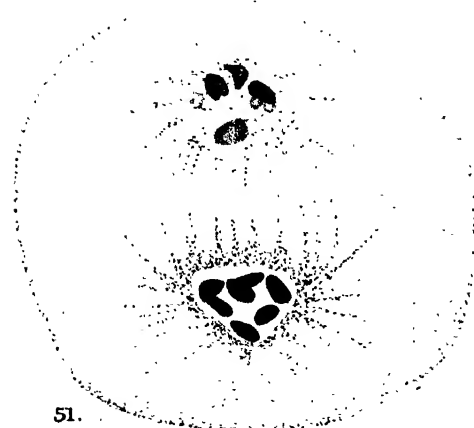
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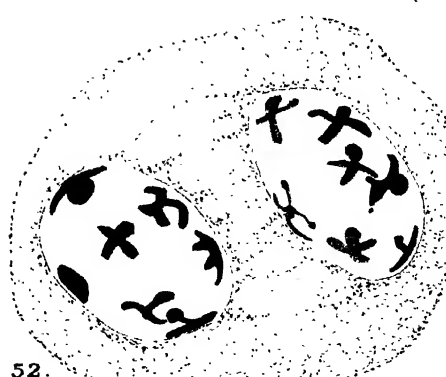
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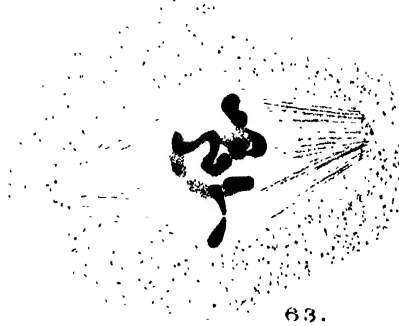
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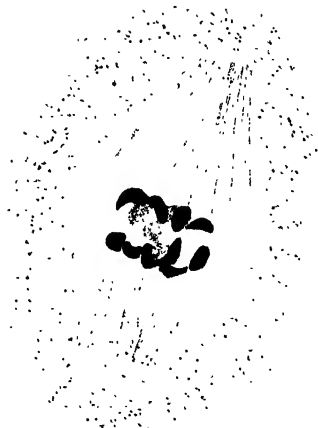
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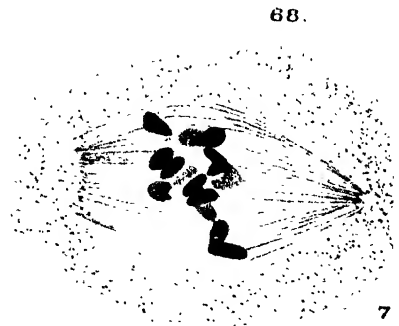
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64.



65.



61b



69.



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NOTE.

NOTE ON THE OCCURRENCE OF PYTHIUM PROLIFERUM, DE BARY, ON THE ROOTS OF THE STRAWBERRY.—*Pythium proliferum*, de Bary, has been described by various observers as being essentially a saprophyte, growing on dead insects in aquatic surroundings, or on vegetable debris in the soil. (See Dr. E. J. Butler, 'Mem. Dept. Agr. India', vol. i, No. 5, 1907.)

At the beginning of July of the present year the writer came across *Pythium proliferum* growing on living roots of the cultivated strawberry in Lanarkshire. During the last number of years an important disease of the roots of the cultivated strawberry has caused great economic loss in the fruit-growing districts of the Clyde Valley. The disease has been diagnosed as being due to unfavourable soil conditions coupled with the attack on the weakened roots by parasitic soil fungi, including the more common species *P. debaryanum*. The latter was found associated with blemished and decayed roots during all the seasons, and was frequently extracted from diseased roots taken from fields where the soil is apt to lie wet.

The specimens of *P. proliferum* were obtained under the following circumstances. At the beginning of July the strawberry plant, whilst ripening its fruit, also undergoes a revival of its vegetative activities, and runners and new roots are thrown out. In one field under observation, where the beds had been severely attacked, plants were lifted for examination of the roots. The field in question was on the top of a hillock with soil of a stiff clay consistency. It should also be mentioned that this area had originally been wooded, as indicated by the numerous tree stumps. During the first fortnight of July the weather was continuously wet, and the soil in the diseased area was more or less water-logged. The newly formed roots were found to be in a blemished state, often most severe in the region of the apical meristems, but also in the older parts of the roots. Such roots, along with more severely damaged ones, were taken into the laboratory, placed in dishes of distilled water, and after one to three days good specimens of *P. proliferum* were found growing out from the blemished and decaying zones. More roots from the same area were treated in this way, and the fungus was found to be of fairly consistent occurrence, not only on old decaying roots, but also on young living roots.

These observations suggest that *P. proliferum* is not only a saprophyte, but also, under certain circumstances, a facultative parasite. In order to attack the roots in question the parasitic powers need not be highly developed. The young roots growing into the soil under the conditions described above are not in a healthy state. They tend to be soft and lax, and in the water-logged state of the soil they suffer from lack of adequate aeration. Their much-weakened state would thus allow of attack by even a comparatively feeble parasite. Given suitable conditions there is no reason to

believe that *P. proliferum* should not behave as such. The ultimate test, of course, is that of pure culture and inoculation experiments. In the meantime the observation of this organism on living roots seems of some importance.

The material examined was found to show a considerable amount of variation, but in the main the character of the mycelium, the sporangia, the liberation of the large zoospores, and the process of proliferation agree with Dr. Butler's account of this interesting species. Certain minor points of difference were observed. By carrying out the culture methods described by the last author the growth of the sporangia and the liberation of the zoospores were observed, and also the movement of the latter and their ultimate germination.

Little is known of the biology of some of these more obscure species of Phycomycetes, and only occasionally can a good supply of material be obtained. Further investigations of this material are being carried on and a more complete account of the author's observations is in progress.

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July 22, 1927.

I. A. R. I. 75.

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